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# Bulletin

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# Torrey Botanical Club

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## Torreya

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BULLETIN OF THE TORREY BOTANICAL CLUB
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# A COMPARATIVE CYTOLOGICAL STUDY OF THREE SPECIES OF THE CHENOPODIACEAE[1]

SISTER MARIE BERNARD WITTE, O.S.F.[2,3]

## INTRODUCTION

While studying root tips of *Spinacia oleracea*, Stomps (1910) observed many cells having 2n, 4n, 8n, and rarely 16n chromosomes. The term polysomaty was given to this phenomenon by Langlet (1927). Polysomaty may be defined as that condition wherein one or more tissues of a diploid organism contain some cells of varying degrees of polyploidy. The polyploid condition arises by double reproduction of the chromosomes and at least one mitotic division always intervenes between two successive double reproductions. The process is indeterminate and continues as long as growth and differentiation occur. The multiple complex cells in *Culex pipiens* described by Berger (1938) can not be considered polysomaty because no mitotic divisions occur between the repeated double reduplications. Polyploidy as found to occur in the shoot and leaf of *Allium cepa* (Berger & Witkus 1946) and in the root tips of *Mimosa pudica* (Witkus & Berger 1947) likewise can not be considered polysomaty because in these cases the condition is determinate and exists for only a definite short period in early development.

Polysomaty has been found in a number of plants many of which belong to the family *Chenopodiaceae*. *Spinacia oleracea*, a member of this family, has been studied several times in order to determine the origin of the polysomatic condition. Gentcheff and Gustafsson (1939) and Berger (1941) have given the most satisfactory and complete explanation of its origin.

As a result of these more recent observations it was suggested that a reinvestigation be made of several species of the *Chenopodiaceae* to determine if polyploid cells originated in the same manner in these plants as in *Spinacia oleracea*. Other cytological phenomena were observed during this reinvestigation and an attempt was made to correlate the histological and developmental structures of the root with the cytological conditions.

There is some confusion in the nomenclature of species, even of some genera, in this family of plants. Two of the species included in this study are listed differently by taxonomists. Some consider *Atriplex patula* and *Atriplex hastata* as two separate species. Others claim that *Atriplex hastata*

[1] The author is indebted to Dr. C. A. Berger for assistance and guidance in this work.

[2] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biology, Fordham University.

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is only a variety of *Atriplex patula*. It is this latter classification which the writer has adopted. Again some taxonomists consider *Kochia trichophylla* as a variety of *Kochia scoparia*, but the majority consider these as two separate species. This confusion in taxonomy may be responsible for the lack of agreement in the chromosome numbers reported for some species of this family. Different numbers have been reported for *Atriplex patula* and *Chrenopodium album*, two species studied in the present work.

## MATERIALS AND METHODS

*Atriplex patula*, *Chenopodium album*, and *Kochia trichophylla* were used in this study. Plants of *Atriplex patula* were collected at Pelham Bay, New York, in late September and transplanted to the greenhouse. Some seeds were removed and dried; others were allowed to fall upon the soil and give rise to new plants the following February and March. Plants of *Chenopodium album* were gathered on the University campus and were likewise transplanted to the greenhouse. Seeds of *Kochia trichophylla* were purchased from Stumpp and Walter Company, New York.

The identifications of *Atriplex patula* and *Chenopodium album* were verified by Dr. H. A. Gleason of the New York Botanical Gardens, whose helpful assistance is most gratefully appreciated.

Primary root tips were obtained by germinating seeds on moist filter paper or moist sand. Secondary root tips were obtained either by digging up plants directly from the soil and cutting uninjured roots or by placing the plants in tap water for a few days and then removing the tips.

Root tips for smears were fixed in the ordinary alcohol–acetic acid fixative and stained with aceto-orcein. Those for sections were placed in Craf fixative and stained in iron-alum haematoxylin. Some sections used for the study of root structure were stained by Foster's tannic acid and iron chloride method. Sections were cut 8 and 10 $\mu$ thick.

## OBSERVATIONS

**Root Structure.** The roots of *Atriplex patula*, *Chenopodium album*, and *Kochia trichophylla* are in general very similar in structure. The roots of *Atriplex patula* were studied most thoroughly and the description given here applies principally to this plant. The other two plants will be described only in so far as they differ from *Atriplex patula*.

Differentiation of the three primary regions, plerome, periblem, and dermatogen, begins approximately 120 $\mu$ from the tip and the three layers are completely established at 150 $\mu$. About 180 $\mu$ from the tip the outer periblem cells enlarge and this enlargement extends posteriorly for approximately 400 $\mu$ to the region where differentiation occurs (figs. 1, 2, 3, 4).

The innermost region of the meristem, the plerome, is composed of a few

large cells surrounded by small tightly packed cells. Few intercellular spaces were observed in this region of the root.

In the periblem there are from three to seven rows of cells. Beginning near the root tip with correspondingly few rows, the number of rows increases gradually until a maximum number is reached at about 400 $\mu$ from the tip. The number of rows of periblem cells is higher in secondary roots than in primary roots while the size of individual cells is greater in primary than in secondary roots. The larger periblem cells are always more distal from the center of the root than smaller cells and are frequently separated from each other by large intercellular spaces. The two inner rows of periblem cells are conspicuous by the fact that their cell walls are often arranged in such a position as to give the appearance of right angles where four cells are in contact. This condition was not so regular in *Chenopodium album* or *Kochia trichophylla* as in *Atriplex patula*.

The dermatogen, which consists of a single layer, has both large and small cells. In this region also the cells of primary roots are larger than those of secondary roots.

In all three plants the meristematic region is quite short. Inner periblem cells could always be found undergoing division farther back in the root tip than middle and outer periblem cells. The meristematic region in *Atriplex patula* is slightly longer than the same region in *Chenopodium album* and *Kochia trichophylla*. In *Atriplex patula* most divisions cease between 500 and 600 $\mu$ while in *Chenopodium album* and *Kochia trichophylla* very few divisions were observed beyond 500 $\mu$.

**Polysomaty.** Different authors had reported 18 and 36 as the diploid number of chromosomes in *Atriplex patula* and 18, 36, and 54 as the diploid number in *Chenopodium album*. The present study showed clearly that 18 is the diploid number in *Atriplex patula* and 36 the diploid number in *Chenopodium album*. A chromosome count of eighteen for *Kochia trichophylla* agreed with all previous reports (figs. 5, 6, 7).

The inner layer of periblem cells in all instances contained only diploid cells. Mitotic divisions were first encountered at about 80 to 90 $\mu$ from the tip. Cells at the apex of the meristematic region were more or less uniform in size and were diploid. About 250 $\mu$ from the tip the periblem cells began to enlarge in the outer layers and from that region on tetraploid cells with paired chromosomes were observed in all three species. In *Atriplex patula* some tetraploids with unpaired chromosomes as well as a few octoploid cells were observed, but no octoploids were seen in *Chenopodium album* or *Kochia trichophylla*. The octoploids always had paired chromosomes and occurred in the next-to-the-outermost row of periblem cells (fig. 8). In the regions where octoploid cells were observed, all cells of the outer layers of the peri-

blem were large. Many of these cells were not in division, but were probably polyploid, tetraploids and octoploids. The cells in this region were greatly vacuolated (fig. 9). Tetraploid cells were also found in the dermatogen layer in *Atriplex patula* and *Chenopodium album* but not in *Kochia trichophylla*.

At prophase in all three species diploid cells showed long single chromosomes scattered throughout the nucleus. Paired tetraploid and octoploid cells showed, at the earliest visible prophase, long relationally coiled pairs of chromosomes (figs. 10, 11, 12, 13). These paired chromosomes were separated throughout their entire length. In *Chenopodium album* few cells underwent a double reduplication. In this species cells which gave rise to the polyploid condition had a prolonged prophase stage and only a few polyploid metaphases were found.

Figure 14 shows a large cell from *Atriplex patula* with the metaphase chromosomes closely paired. In a few instances (fig. 15) the paired chromosomes still showed their close association at early anaphase. No active pairing of chromosomes at late anaphase or early telophase was observed.

Since polysomaty had previously been reported for *Kochia trichophylla* the present study on this species was concerned with the origin of polyploidy and a comparison of the details of the process with that found in *Atriplex patula*, *Chenopodium album*, and *Spinacia oleracea*. In *Kochia trichophylla* there were considerably more cells in the early paired prophase stage than had been observed in *Chenopodium album*. The early paired prophase condition was also much clearer in *Kochia trichophylla* than in *Chenopodium album*.

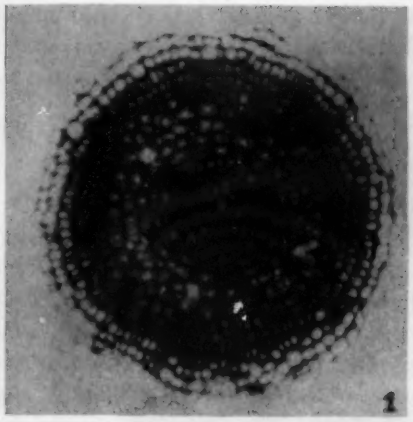
In all three species polysomatic divisions were found in old as well as in young roots, both primary and secondary.

**Early Separation of Chromatids in Kochia trichophylla.** Frequently at late prophase the chromatids in *Kochia trichophylla* were some distance apart (fig. 16). That this precocious separation of chromatids was not due to excessive pressure in smearing was shown by the fact that it was likewise visible in sectioned material.

Prior to reaching the metaphase plate the chromatids were completely separated so that at metaphase each chromatid could be seen lying close to, but separated from, its sister chromatid. Figure 16 shows the spindle-attachment regions clearly separated. Other cases were observed in which the

**Explanation of figures 1–7**

FIGS. 1–4, photomicrographs of cross sections of root tips of *Atriplex patula* taken respectively at 180, 240, 300, and 360 μ from the tip. Magnification ×200. FIGS. 5–7, ×1500. FIG. 5, late prophase diploid cell of *Atriplex patula*, aceto-orcein smear preparation. FIG. 6, late prophase diploid cell of *Chenopodium album*, iron-alum haematoxylin section. FIG. 7, late prophase diploid cell of *Kochia trichophylla*, aceto-orcein smear preparation.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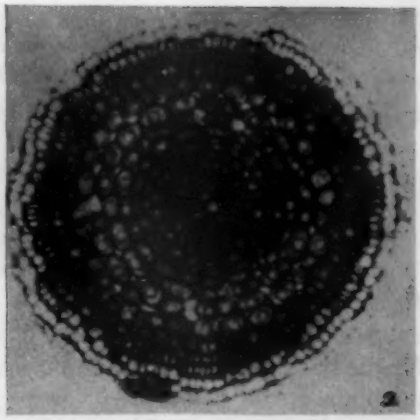
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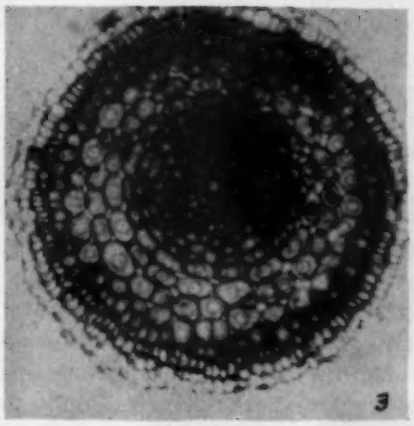
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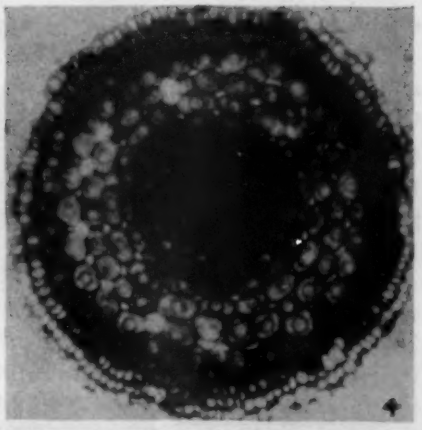
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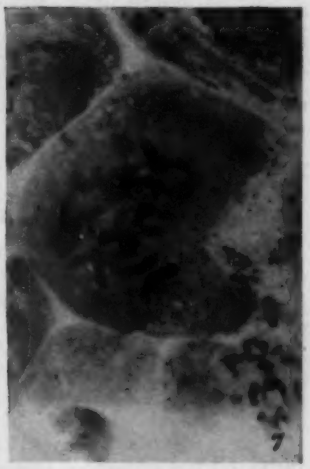
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chromatids were loosely held together at the SA-region as they approached the metaphase plate. This same phenomenon was also observed in tetraploid cells (figs. 17, 18). This would again indicate that this phenomenon is characteristic of *Kochia trichophylla*.

## DISCUSSION

Various methods have been proposed to explain the origin of polysomatic cells. Breslawetz (1926), Huskins and Smith (1932), Meurman (1933), and Wulff (1936) suggested that polyploidy was due to a type of cell or nuclear fusion. A second hypothesis was given by Stomps (1910), de Litardière (1923), and Hardh (1939), in which the chromosomes undergo a double longitudinal split during the prophase stage. Wulff (1940) explained the doubling in the *Aizoaceae* by way of a "double longitudinal splitting" or "inner division," and Lorz (1937) stated that the chromosomes divide and separate at a faster rate than the nucleus as a whole.

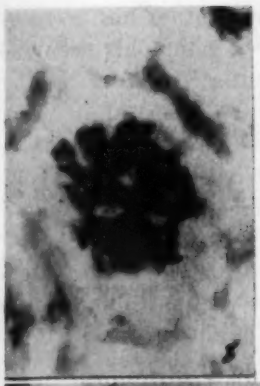
The foregoing observations on *Atriplex patula*, *Chenopodium album*, and *Kochia trichophylla* further substantiate the evidence of Gentcheff and Gustafsson (1939), Berger (1941), and Ervin (1941), that polysomaty arises by a double chromosome reproduction during the resting stage. The paired, relationally coiled chromosomes were clearly visible at very early prophase, which signifies that the double reduplication had occurred somewhat before this time and therefore during the resting stage.

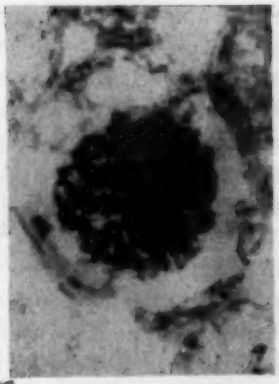
In *Spinacia* the sequence of polyploid cells is as follows: 2n unpaired—4n paired—4n unpaired—8n paired—8n unpaired—16n paired—and rarely 16n unpaired. In the species here reported on it is thought that as soon as the periblem cells have begun to enlarge all of the outer periblem cells become tetraploid. If these cells undergo division, the first mitosis after double reduplication will give prophase and metaphase stages with the chromosomes rather closely associated. Any division following this will have chromosomes which are relatively less associated, the pairing being disturbed by the first mitosis.

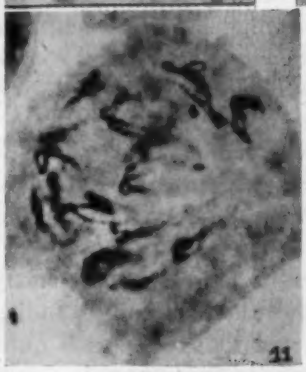
There are undoubtedly more octoploid cells present in the root tips of *Atriplex patula* than were observed in division, but since these cells become

**Explanation of figures 8–18**

FIGS. 8 and 9 from iron-alum haematoxylin sections. FIGS. 10–18 from aceto-orcein smear preparations. Magnification of all figures ×1500. FIGS. 8–11 from *Atriplex patula*. FIG. 8, Paired octoploid metaphase periblem cell. FIG. 9, Paired octoploid late prophase cell of the periblem. FIG. 10, Paired tetraploid early prophase stage. FIG. 11, Paired tetraploid early prophase stage (somewhat earlier than fig. 10). FIG. 12, Tetraploid early prophase stage of *Chenopodium album*. FIG. 13, Paired tetraploid early prophase stage of *Kochia trichophylla*. FIG. 14, Paired tetraploid metaphase stage of *Atriplex patula*. FIG. 15, Paired tetraploid anaphase of *Atriplex patula*. FIG. 16, Diploid metaphase plate of *Kochia trichophylla* with chromatids separated. FIGS. 17, 18, Tetraploid metaphase plates of *Kochia trichophylla*.







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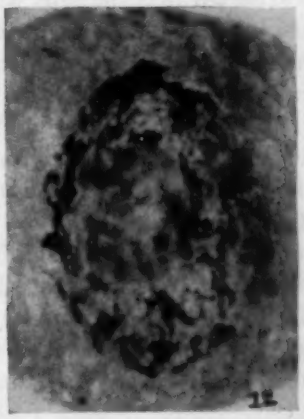

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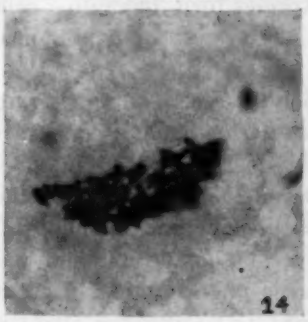

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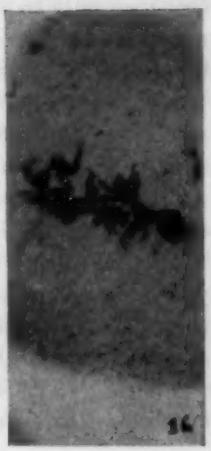

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18

polyploid only after a considerable degree of differentiation has occurred very few of them undergo division. Since only paired octoploids occurred, it can be concluded that these cells become differentiated before another division can occur. While no octoploid divisions were observed in either *Chenopodium album* or *Kochia trichophylla* this does not denote a complete absence of such cells. Wulff (1936) investigated *Kochia scoparia* and *Kochia trichophylla* and found tetraploid cells with 36 chromosomes and octoploids with 72.

Since the meristematic region is shorter in *Chenopodium album* and *Kochia trichophylla* than in *Atriplex patula* it seems very possible that the degree of polyploidy reached in a particular species might be related to the length of the meristematic region. If the region of active division is very short then it would be expected that the cells would not have sufficient time to undergo many double reduplications. In *Chenopodium album* and *Kochia trichophylla* no octoploid cells were observed. These two species have a short meristematic region and the cells differentiate before they have an opportunity to become octoploid. In *Atriplex patula*, with its longer meristematic region, some cells have sufficient time to undergo a second double reduplication. In *Spinacia* the long meristematic region offers a good explanation of the high degree of polyploidy attained.

This interpretation not only explains the different degrees of polysomaty found in different species but offers a useful suggestion for understanding the difference between polysomatic and non-polysomatic species. It is quite possible that many supposedly non-polysomatic species form tetraploid cells which differentiate before they have an opportunity to reveal their polyploid condition by mitosis.

In *Atriplex patula* and *Chenopodium album* polyploid cells were observed not only in the periblem region but also in the dermatogen. Polysomaty occurred only in the periblem of *Kochia trichophylla*. Ervin's (1941) statement, that he considered the condition in *Cucumis melo* as apparently similar to that in various members of the *Chenopodiaceae* and that the main difference appeared to be that in the *Chenopodiaceae* the dermatogen was wholly monosomatic whereas in *Cucumis melo* some polysomatic cells were usually present in the dermatogen, needs, therefore, to be modified slightly. At least in some members of the *Chenopodiaceae* the dermatogen is not entirely monosomatic.

Ervin (1941) reported a paired condition of chromosomes during anaphase movement similar to that observed occasionally in *Atriplex patula*. In these cases of prolonged association the pairing is lost during later anaphase and telophase so that at the next division the chromosomes are unpaired.

Wulff (1936) remarked that in the genus *Chenopodium*, the polyploid species, with the exception of *C. Bonus-Henricus*, showed polysomaty; the

diploid species did not show this phenomenon. Since 36 chromosomes were determined as the diploid number for *C. album*, this plant is a polyploid species. As this species contains polysomatic tissue it thus supports Wulff's conclusion that only polyploid species of *Chenopodium* show polysomaty.

The separation of chromatids prior to their alignment on the metaphase plate as observed in *Kochia trichophylla* might perhaps be explained by a special condition of the matrix. In *Kochia trichophylla* the matrix apparently loses its ability to hold the chromatids together much earlier than it does in the majority of plants, where the separation does not occur until the chromosomes are at the equatorial plate, and where separation begins at the SA-region and then progresses terminally. It is generally known that chromatids usually come to lie even closer together at metaphase, so that they frequently appear as a single structure.

A phenomenon very similar to the early separation of chromatids described here has been reported by Grell (1945, 1946) in *Culex pipiens*. In *Culex* Grell has described four types of prometaphase chromosomes in which a more or less continuous series ranged from a condition in which the SA-regions were undivided and chromatid arms were partially or completely in contact to one in which both SA-regions and chromatid arms were completely separated and lay parallel to each other. That these paired, parallel bodies were actually chromatids was demonstrated by their subsequent behavior; the members of each such pair went to opposite poles at anaphase. According to Grell's classification the prometaphase chromosomes of *Kochia trichophylla* could be considered belonging to the fourth type.

## SUMMARY

1. The root structure of *Atriplex patula*, *Chenopodium album*, and *Kochia trichophylla* is described. The meristematic region in *Atriplex patula* is slightly longer than the same region in *Chenopodium album* and *Kochia trichophylla*.

2. Diploid cells of *Atriplex patula* contain 18 chromosomes while diploid cells of *Chenopodium album* have 36 chromosomes. The chromosome number of 18 found in *Kochia trichophylla* agrees with previous reports.

3. Polysomaty occurs in the periblem and dermatogen of *Atriplex patula* and *Chenopodium album* and in the periblem of *Kochia trichophylla*.

4. Polysomaty in the three plants investigated, is brought about by a double reduplication in the resting stage, as was earlier reported for *Spinacia oleracea*.

5. The inner periblem cells are always diploid, while polyploid cells are present in the outer layer of the periblem.

6. The proportionately large number of early paired prophase stages as compared with the number of later prophase and metaphase stages indicates a prolonged early prophase condition in tetraploid cells.

7. It is suggested that the length of the meristematic region may be a factor in determining the degree of polyploidy reached. *Atriplex patula*, having a slightly longer meristematic region, contains some octoploid cells.

8. The chromatids in *Kochia trichophylla* separate before reaching the metaphase plate. This condition is observed in diploid and tetraploid cells.

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# SOMATIC CHROMOSOMES OF PEDIGREED HYBRID PETUNIA

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## INTRODUCTION

In the spring of 1946 the author was offered the opportunity of studying the somatic chromosomal complements of the members of a pedigreed hybrid population of *Petunia*.[2] Genetical breeding experiments on this population have been in progress for fifteen years with a view to determine the various types, nature, genetics, and effects of the different classes of both intraspecific and interspecific sterilities.

The present report is limited mainly to the determination of the chromosome numbers occurring in the progenies of 4n *Petunia axillaris* × 2n *P. axillaris* and of 4n *P. axillaris* × 2n *P. integrifolia*. The analysis of these progenies from the viewpoint of the specialized factors operating in fertility and sterility and the description of the morphology and growth habits lie within the scope of a forthcoming publication of Dr. A. B. Stout.

The present report has certain definite advantages over preceding reports of chromosome numbers in progenies of crosses in *Petunia*. The progenies here reported upon were derived from crosses of two pure species of *Petunia* and the range of crosses embraces higher-polyploid forms than had been previously reported for this genus.

The reports on naturally occurring polyploid races in the plant kingdom, on the experimental production of polyploid forms and on crosses involving polyploids have produced a vast amount of literature. The present paper is a contribution to the general problem of polyploidy and serves more particularly as a cytological basis for the interpretation of fertilities and sterilities in *Petunia*.

## MATERIAL AND METHODS

**Primary Species of the Population of Petunia.** *Petunia integrifolia* (Hook.) Schinz & Thellung (1915) has small "violet" flowers and is very different from the two other primary species described below. Every individual of this species is self-incompatible. The use of the binomial *P. integ-*

[1] Work done under the direction of Dr. C. A. Berger, S.J., head of the Department of Biology at Fordham University, submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in that Department.

[2] The author is indebted to Dr. A. B. Stout of the New York Botanical Garden both for the use of the material reported upon and for several communications concerning the taxonomy of the species under investigation.

*rifolia* in preference to *P. violacea* is in accordance with the present rules of international nomenclature according to the findings of Stout (personal communication).

*P. axillaris* (Lam.) B.S.P. (*P. nyctaginiflora* Juss.) is very distinct, with white flowers and other clearly differentiated characteristics. Every individual plant of the species is self-incompatible according to Stout and Chandler (1941, 1942) and Stout (1945).

*P. parodii* Steere (1931) is somewhat similar to *P. axillaris* but quite distinct and always fully self-compatible.

**Tetraploid Forms of P. axillaris.** Diploid plants of *P. axillaris* were treated with colchicine by Stout and Chandler, (1941, 1942) and a number of tetraploid forms were obtained.

**Tetraploid-diploid Crosses and Progenies.** All the progenies studied in this work were derived from the two crosses, 4n *P. axillaris* × 2n *P. integrifolia*, and 4n *P. axillaris* × 2n *P. axillaris*. The progenies are listed below as series and the parentages are indicated with the seed parent listed first in each cross.

*Series 170.* The parents of this series are 4n *P. axillaris* × 2n *P. integrifolia.*
*Series 170–10.* This is a clone derived from a branch of 170–4 which had been treated with colchicine. Each member of the clone is termed a *ramet* (Stout 1929). Ten ramets were examined.
*Series 217.* The parents are two ramets of 170–10.
*Series 224,* The parents are two ramets of 170–10.
*Series 227.* The parents are two ramets of 170–10.
*Series 245.* The parents are 139–12T 4n *P. axillaris* × 2n *P. axillaris.*
*Series 246.* The parents are 172–28 4n *P. axillaris* × 2n *P. axillaris.*
*Series 247.* The parents are 139–12T 4n *P. axillaris* × 2n *P. axillaris.*
*Series 235.* The parents are 170–3 × 2n *P. integrifolia.*
*Series 238 and 239.* The parents are 170–1 × 2n *P. integrifolia.*
*Series 242.* The parents are 170–4 × 139–12 2n *P. axillaris.*
*Series 243.* The parents are 170–5 × 139–12 2n *P. axillaris.*
*Series 230.* The parents are 170–3 × 170–1.
*Series 318.* The parents are 170–3 × 170–5.
*Series 319.* The parents are 170–7 × 170–1.
*Series 320.* The parents are 170–4 × 170–10.
*Series 321.* The parents are 170–10 × 170–10.
*Series 241.* The parents are 170–7 × 139–12T 4n *P. axillaris.*
*Series 266.* The parents are 170–5 × 171–28 4n *P. axillaris.*
*Series 279.* This series includes the progeny from the selfing of 217–4.
*Series 280.* This series includes the progeny from the selfing of 217–5.
*Series 281.* This series includes the progeny from the selfing of 217–18.
*Series 282.* This series includes the progeny from the selfing of 224–5.
*Series 283.* This series includes the progeny from the selfing of 227–2.
*Series 284.* This series includes the progeny from the selfing of 224–1.
*Series 285.* The parents are 217–4 × 139–12T 4n *P. axillaris.*
*Series 286.* The parents are 217–12 × 171–28 4n *P. axillaris.*
*Series 287.* The parents are 222–6 4n *P. axillaris* × 224–5.

**Cytological Methods.** The examination of the chromosomal complements of the various members of the population of *Petunia* was made by the

leaf smear technique of Baldwin (1939) in union with the Feulgen stain (Meyer 1943). The fixation period was 1–2 hours in 3:1 absolute alcohol–glacial acetic acid. The young leaves surrounding a young vigorous flower bud were found most suitable. After fixation, the young leaves were rinsed in tap water for 1–5 minutes and then hydrolyzed in N HCl at 55–60° C for 10 minutes. The hydrolyzed leaves were rinsed in tap water for 1–5 minutes and then placed in a vial of decolorized fuchsin for 20–30 minutes. The entire stained leaves were then placed in 45 % acetic acid and the basal third of the leaf, in which most mitotic activity is present according to Speese (1939), was cut into pieces of approximately 0.5 mm. The use of 10 % acetic in place of the 45 % acetic was found to allow greater pressure in the subsequent smearing. Forceful pressure by means of a matchstick on the cover glass produced flat, well spread figures.

The temporary smears were made permanent according to the method of McClintock (1929) with the following modifications: the temporary smears were left overnight in a moist chamber containing equal parts of 45 % acetic acid and 95 % alcohol, the fluid barely touching the lower edge of the cover slip. On the following day a razor blade was gently inserted beneath the edge of the cover slip and the slip lifted off. The smear almost invariably adhered to the cover slip. The cover slip was then run through two changes of 95 % alcohol and combined with a new slide in Diaphane. A counter-stain of orange G or light green was frequently added in the first 95 % alcohol.

*Pretreatments.* All the leaf smears were pretreated. Burrell (1939) had suggested the use of colchicine to contract chromosomes as a pretreatment for root-tip smears. Meyer (1943) used this colchicine pretreatment in conjunction with the leaf smear technique and Feulgen stain. The scarcity of colchicine forced the author to use a variety of substitutes in the pretreatment of the leaf smears.

Several substitutes employed by the author had been reported previously in the literature, namely, paradichlorobenzene (Meyer 1945), acenaphthene (Swanson 1940), and chloral hydrate, which had had a long history since the work of Němec (1904) and has been employed in particular by Swanson (1943).

Pretreatment with p-dichlorobenzene was relatively unsuccessful although many attempts were made. There was generally only a very slight contraction of the chromosomes. In some cases contraction did occur and it was possible to spread the chromosomes but the chromosomes lacked clear cut outlines in these smears (fig. 12). The results with acenaphthene were similar except that the chromosomes had somewhat clearer outlines.

The action of chloral hydrate was so toxic that it was not found possible to obtain any accurate counts of chromosomes. The abnormal condition of the

excised leaves may have accentuated the toxic effects. Dividing cells were very rare and contraction, when it was observed, showed the chromosomes as mere "blocks."

In the fall of 1946, Dr. H. Wechsler brought to the attention of the author a reference to the work of King and Sullivan (1946) on the similarity of action of podophyllin and colchicine. The present author undertook a rapid test of the action of podophyllin on excised root tips of *Allium cepa* and on young excised leaves of *Petunia*. Sullivan and Wechsler (1947) reported the c-mitotic activity of podophyllin on growing root tips of *Allium cepa*.

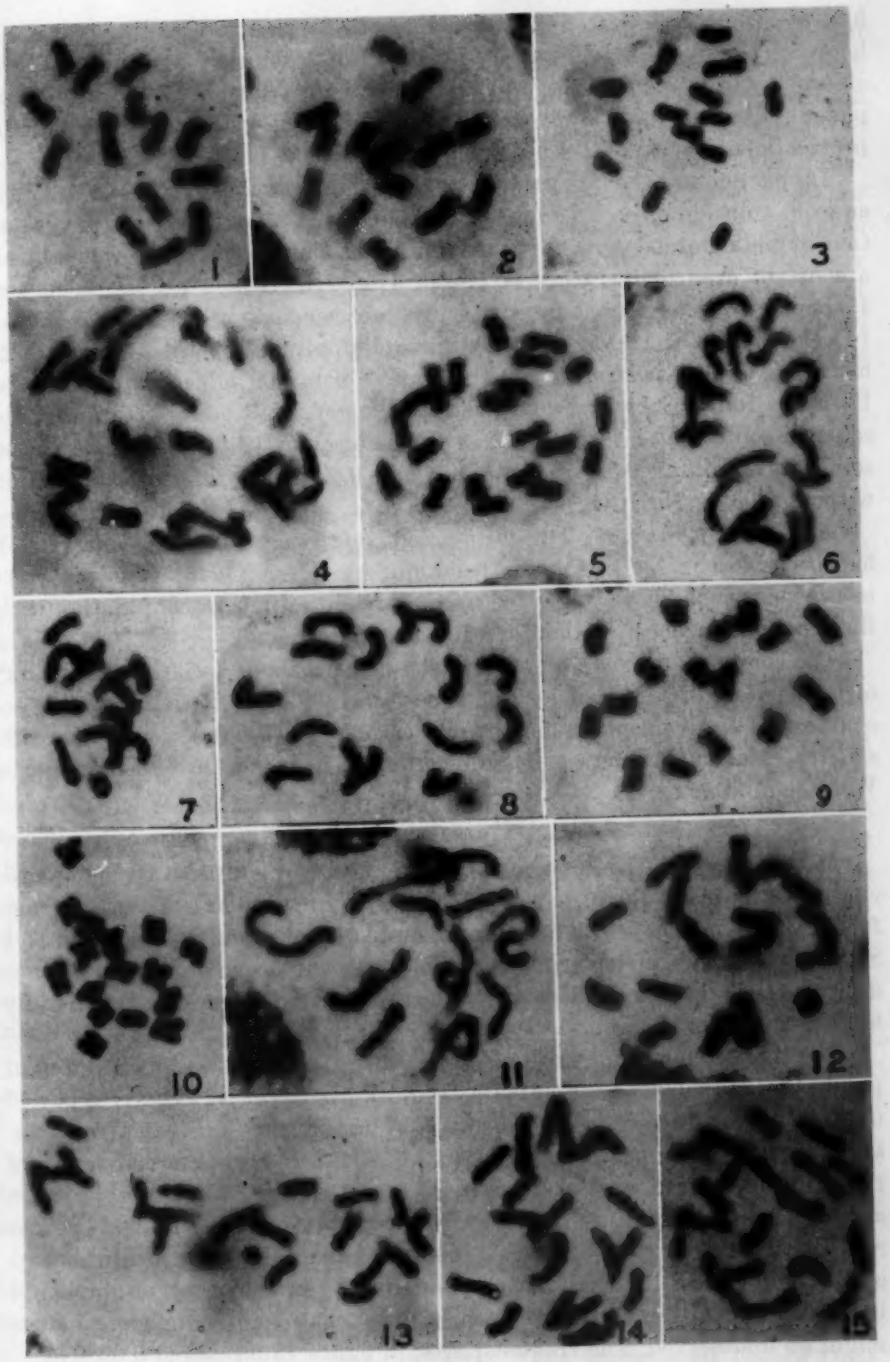
The pretreatment with podophyllin consisted of 1 g. of podophyllin in 100 ml. of tap water, thoroughly shaken and allowed to stand overnight. The mixture was filtered and then used for the pretreatment of the young excised leaves.

The contracting effect of podophyllin alone was very similar to that of p-dichlorobenzene and acenaphthene but the number of dividing cells seemed much greater after the pretreatment with podophyllin. The degree of contraction was very slight even after prolonged pretreatment and so this agent alone did not prove to be of great value in counting small chromosomes. Some good preparations were obtained (figs. 15, 16).

The pretreatment with podophyllin followed by chloral hydrate produced many good figures that spread well. The most useful combination was a 2-hour pretreatment of podophyllin followed by 3 hours of a 0.06 % chloral

**Explanation of figures 1–15**

FIGS. 1–15. Photomicrographs of *Petunia* leaf smears. Magnification × 2352. FIG. 1. Feulgen leaf smear of diploid (14) *P. axillaris*. Pretreatment: 2 hours of 1% podophyllin followed by 3 hours of 0.06% chloral hydrate; this same pretreatment used on material shown in figures 2, 3, 4, 5, and 6. FIG. 2. Aceto-orcein leaf smear of diploid (14) *P. integrifolia*. FIG. 3. Aceto-orcein leaf smear of diploid (14) *P. parodii*. FIG. 4. Feulgen leaf smear of 4n (28) *P. axillaris*. FIG. 5. Feulgen leaf smear of a triploid (21) from a cross 4n *P. axillaris* × 2n *P. integrifolia*. This plant was the source of the colchicine induced hexaploids. FIG. 6. Feulgen leaf smear of a hypotriploid (20) segregate from a cross 4n *P. axillaris* × 2n *P. integrifolia*. FIG. 7. Feulgen leaf smear of a diploid (14) segregate from a cross of hybrid 3n (21) × 2n *P. axillaris*. Pretreatment: 3 hours of a solution containing 0.5 g. podophyllin and 0.05 g. chloral hydrate. The same pretreatment was used on material shown in figures 8, 10, and 13. FIG. 8. Feulgen leaf smear of a trisomic (15) segregate from a cross of hybrid 3n (21) × 2n *P. axillaris*. FIG. 9. Feulgen leaf smear of a polysomic (16) segregate from a cross of a hybrid 3n (21) × 2n *P. integrifolia*. Pretreatment: 1.5 hours of podophyllin followed by 1.5 hours of 0.1% chloral hydrate. This same pretreatment was used on material shown in figures 11 and 14. FIG. 10. Feulgen leaf smear of a polysomic (16) segregate from a cross of hybrid 3n (21) × 2n *P. axillaris*. FIG. 11. Feulgen leaf smear of a polysomic (17) segregate from a cross of hybrid 3n (21) × 2n *P. integrifolia*. FIG. 12. Feulgen leaf smear of a polysomic (18) segregate from a cross of hybrid 3n (21) × 2n *P. integrifolia*. FIG. 13. Feulgen leaf smear of a polysomic (19) containing a fragmented chromosome from a cross of hybrid 3n (21) × 2n *P. axillaris*. FIG. 14. Feulgen leaf smear of a hypotriploid (20) segregate from a cross of hybrid 3n (21) × 4n (28) *P. axillaris*. FIG. 15. Feulgen leaf smear of a polysomic (19) segregate from a cross of two hybrid triploids. Pretreatment: 3 hours of podophyllin.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hydrate (figs. 1, 2, 3, 5, 6, 20). Good results were also obtained using 1.5 hours of podophyllin followed by 1.5 hours of a solution containing 0.1 % chloral hydrate (figs. 4, 14, 27, 28). The single solution containing 0.5 g. podophyllin and 0.05 % chloral hydrate was used with good success in a pretreatment period of 3–4 hours (figs. 4, 8, 10, 13, 17, 21, 23, 25).

In particular, the following pretreatments were used: (1) a 0.05 % aqueous solution of colchicine for 3–4 hours; (2) a saturated aqueous solution of p-dichlorobenzene prepared the night before and left overnight at a constant oven temperature of 55–60° C and used as a pretreatment for 1.5 hours; (3) a saturated aqueous solution of acenenaphthene for 3.5 hours; (4) the filtrate of 1 g. of podophyllin in 100 ml. of tap water for 1.5 to 3 hours; (5) the filtrate of 1 g. of podophyllin in 100 ml. of tap water for 1.5 hours followed by a second pretreatment of 1.5–3 hours of an aqueous solution containing either 0.06 % or 0.1 % of chloral hydrate; (6) a single solution containing 0.5 g. podophyllin and 0.05 g. chloral hydrate with or without 0.06 % $NaHCO_3$.

*Aceto-orcein smears.* The aceto-orcein smear method of LaCour (1941) had been used in the leaf smear technique by Rösen (1946). The pretreatments mentioned above were used by the present author in conjunction with the aceto-orcein smear method in a few instances.

The counts were made by observation of permanent smears with the aid of a fluorite oil-immersion lens of a power of 98× in conjunction with an ocular of 15×. All the photomicrographs except one (fig. 26) were made with a Leitz "Makam" camera of magnification of 1× using an 8× ocular and a Wratten M filter no. 58.

## OBSERVATIONS AND RESULTS

**Morphology of Chromosomes.** The length of the untreated metaphase chromosomes in all three primary species is quite similar and has a range from 2.5 to 4.5$\mu$. The width is approximately 0.6$\mu$. The only observation of a SAT-chromosome in the primary diploid species was in *P. axillaris*. It is difficult to observe the SAT-chromosome as the satellited region is generally hidden and is quite minute.

The measurements of treated chromosomes were made in 52 different plant smears that represent plants from all the types of crosses reported upon. The range of length and breadth of the various degrees of contraction extended from $5.4 \times 0.6\mu$ to $1.26 \times 1.0\mu$. It is easily seen that many of the figures measured were prophases. Figure 11 represents a prophase stage, as can be seen from the uncontracted chromosomes, and this may be the reason for the clarity of the SAT-chromosome seen there. The measurements of chromosomes in the various polyploid forms seem to indicate no change in size but since the chromosomes are quite small a slight change in size might go unobserved. The fragment seen in figure 13 measured $0.6 \times 0.6\mu$.

**Somatic Chromosome Numbers.** The diploid number of all species of *Petunia* has been reported as 14 except for the species *P. parviflora* which has the number 18 (Ferguson & Coolidge 1932; Steere 1932). Ferguson (1924) reported the somatic count of 14 for diploid bedding petunias and the same number was reported for true breeding *P. violacea* Lindl. (Ferguson & Coolidge 1932). Numerous reports of the diploid number of 14 for varieties classified as *P. violacea* have been made and are listed by Ferguson and Coolidge. Ferguson (1928) reported the diploid number of 14 for pure *P. axillaris* and this was confirmed by Steere (1932). The diploid number of 14 was reported by Steere (1931) for *P. parodii* in his description and determination of that new species of *Petunia*. The present author confirms the counts for the three species. Unexpectedly, a fragment was observed in one of the diploids of *P. parodii*. The chromosome complements of the three primary species are seen in figures 1, 2, and 3.

*Tetraploid P. axillaris.* Seven determinations of the originally induced tetraploids and their progeny were made. All plants were exact tetraploids. This is a confirmation of the more extensive meiotic counts of Stout and Chandler (1941). One tetraploid complement is seen in figure 4.

*Segregates from 4n P. axillaris × 2n P. integrifolia.* All seven segregates were determined as exact triploids with a count of 21, except plant no. 170–6 which is seen in figure 6 with a complement of 20 chromosomes. A typical count of the triploid hybrids is seen in figure 5.

*A colchicine-treated triploid.* The triploid hybrid 170–4 was treated with colchicine in order to induce the hexaploid condition. The only ramets available to the author were basal cuttings, which were unaffected by the colchicine treatment. There is no doubt from the evidence given below that the upper cuttings had been changed to the hexaploid condition. It was to be expected that part of the treated plant might remain unaffected by the treatment from the work of Blakeslee and Warmke (1940).

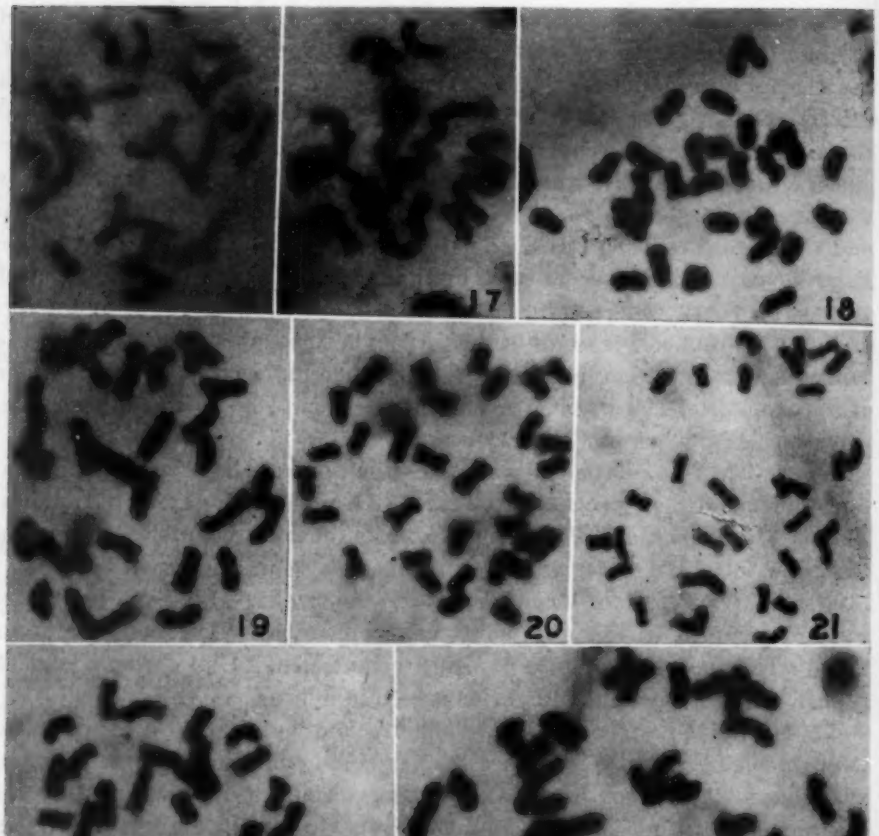
*Tetraploid P. axillaris × 2n P. axillaris.* Four plants were selected at random from the progeny of this cross and all were determined to be exact triploids (21).

*Hybrid triploid 170–3 × 2n P. integrifolia.* Only the classes 15 (trisomic), 16, and 17 were found in the progeny of this 3n × 2n cross (table 1). Typical counts are seen in figures 9 and 11.

*Hybrid triploid 170–1 × 2n P. integrifolia.* All the chromosome classes from diploid to hypotriploid (20) were found in the progeny of this 3n × 2n cross (table 1). A photomicrograph of a typical count is seen in figure 12.

*Hybrid triploid 170–4 × 2n P. axillaris.* All chromosome classes from 15 (trisomic) to 20 were found in the progeny of this 3n × 2n cross. Typical counts are seen in figures 8, 10, and 13. A fragment can be seen in figure 13.

*Hybrid triploid 170–5 × 2n P. axillaris.* The chromosome classes from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diploid (14) to 20 with the exception of classes 17 and 18 were found in the progeny of this 3n × 2n cross. Figure 7 shows a diploid (14) segregate from this cross.

TABLE 1. *Chromosome counts from pedigreed 3n × 2n crosses.*

| Seed parent | Pollen parent | Chromosome numbers | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | |
| 170–3(3n) × 2n | *P. integrifolia* | | 4 | 6 | 2 | | | | | 12 |
| 170–1(3n) × 2n | *P. integrifolia* | 1 | 6 | 1 | 1 | 3 | 1 | 3 | | 16 |
| 170–5(3n) × 2n | *P. axillaris* | 3 | 1 | 1 | | | 1 | 1 | | 7 |
| 170–4(3n) × 2n | *P. axillaris* | | 1 | 1 | 1 | 2 | 1f[a] | 4 | | 10 |

[a] Fragment.

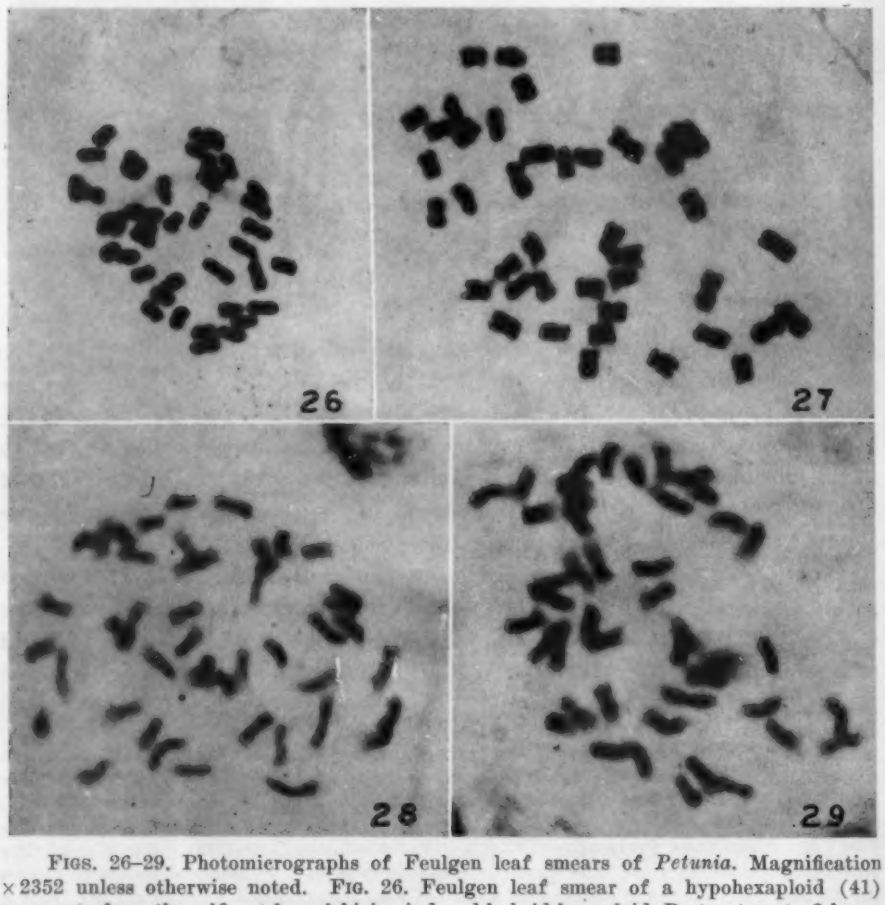
*Hybrid triploid crosses.* The specific triploid-triploid crosses and the somatic chromosome determinations of the members in their progenies are listed in table 2. Of the 10 chromosome classes from 19 to 28 (tetraploid), 7 chromosome classes are represented. There is an apparent concentration in the chromosome class 20. The chromosome classes 19, 20, and 23 have not hitherto been reported for triploid crosses in *Petunia*. Counts of the classes 19 and 25 are seen in figures 15 and 16 respectively.

*Triploid-tetraploid crosses.* The cross of 170–5 (3n) × 4n *P. axillaris* yielded only one plant, with the count of 20 chromosomes. A similar cross of 170–7 (3n) × 4n *P. axillaris* also yielded only one plant, which had a count of 26 chromosomes (fig. 17).

**Explanation of figures 16–25**

FIGS. 16–25. Photomicrographs of Feulgen leaf smears of *Petunia*. Magnification × 2352 unless otherwise noted. FIG. 16. Feulgen leaf smear of a polysomic (25) segregate from a cross of two hybrid triploids. Pretreatment: 3 hours of 1% podophyllin. FIG. 17. Feulgen leaf smear of a polysomic (26) segregate from a cross of hybrid triploid (21) × 4n (28) *P. axillaris*. Pretreatment: 3.5 hours of a solution containing 0.5 g. podophyllin and 0.05% chloral hydrate. The same pretreatment was used on material shown in figures 21, 23, and 25. FIG. 18. Feulgen leaf smear of a hypertetraploid segregate (29) from a cross of hybrid hexaploid (42) × 4n (28) *P. axillaris*. Pretreatment: 1.5 hours of podophyllin followed by 1.5 hours of 0.1% chloral hydrate. The same pretreatment was also used on material shown in figures 19, 22, and 24. FIG. 19. Feulgen leaf smear of a polysomic (31) segregate from a hybrid hypohexaploid (41) × 4n (28) *P. axillaris*. FIG. 20. Feulgen leaf smear of a polysomic (32) segregate from a cross of a hybrid hexaploid (42) × 4n (28) *P. axillaris*. Pretreatment: 2 hours of 1% podophyllin followed by 3 hours of 0.06% chloral hydrate. FIG. 21. Feulgen leaf smear of a polysomic (33) segregate from a cross of a hybrid hypohexaploid (41) × 4n (28) *P. axillaris*. Magnification × 1715. FIG. 22. Feulgen leaf smear of a polysomic (34) segregate from a cross of a hybrid hexaploid (42) × 4n *P. axillaris*. FIG. 23. Feulgen leaf smear of a pentaploid (35) segregate from a cross of a hybrid hexaploid (42) × 4n (28) *P. axillaris*. FIG. 24. Feulgen leaf smear of a hyperpentaploid (36) segregate from a cross of a hybrid hexaploid (42) × 4n (28) *P. axillaris*. FIG. 25. Feulgen leaf smear of a hypohexaploid (40) segregate from the selfing of an colchicine-induced hexaploid.

*Hexaploid progenies.* The hybrid triploid 170–4 had been subjected to colchicine treatment to induce hexaploidy (Stout, personal communication). Unfortunately, none of the original cuttings was available to the author for




FIGS. 26–29. Photomicrographs of Feulgen leaf smears of *Petunia*. Magnification ×2352 unless otherwise noted. FIG. 26. Feulgen leaf smear of a hypohexaploid (41) segregate from the selfing of a colchicine-induced hybrid hexaploid. Pretreatment: 2 hours of 0.06% colchicine. Magnification ×1568. FIG. 27. Feulgen leaf smear of a balanced hexaploid (42) segregate from the selfing of a colchicine-induced hexaploid. Pretreatment: 1.5 hours of 1% podophyllin followed by 1.5 hours of 0.1% chloral hydrate. The same pretreatment was also used in the material shown in figure 28. FIG. 28. Feulgen leaf smear of a hyperhexaploid (43) segregate from the selfing of a hybrid hexaploid. FIG. 29. Feulgen leaf smear of a hyperhexaploid (44) segregate from hybrid hypohexaploid (42) ×4n (28) *P. axillaris*. Pretreatment: 1.5 hours of 1% podophyllin followed by 1.5 hours of 0.1% chloral hydrate.

determination. However, the plants grown from these treated cuttings were selfed and 25 plants were examined for chromosome counts: 16 were exact hexaploids, 6 had 40 chromosomes, and 3 fell into the 41 class (table 3).

TABLE 2. *Chromosome counts from pedigreed 3n × 3n crosses.*

| Seed parent | Pollen parent | Chromosome numbers | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
| 170–3 (3n) × 170–1 (3n) | | 1 | 3 | | | | | 1 | | | |
| 170–3 (3n) × 170–5 (3n) | | | | | | 1 | | | | | |
| 170–7 (3n) × 170–1 (3n) | | | 1 | | | | 1 | 2 | | | |
| 170–4 (3n) × 170–10 (3n) | | | | | | | | | 1 | | |
| 170–10 (3n) × 170–10 (3n) | | | | | | 1 | | | | | 1 |
| | | 1 | 4 | | | 2 | 1 | 3 | 1 | | 1 |

These derived hexaploids were in turn selfed and a large progeny was obtained. Fifty plants were examined: 26 plants had exact hexaploid complements (42), 10 were in the 40 chromosome class, 7 were in the 43 class, 6 in the 42 class, and 1 in the 44 class (table 3). Typical complements of the chromosome classes 40 to 44 are seen in figures 25–29 respectively.

*6n × 4n crosses and the reciprocal.* The cross of 217–4 (41) × 4n *P. axillaris* yielded a large progeny, of which 16 plants were examined at random. The chromosome classes from 29 to 35 were represented with the exception of class 30 (table 4). The similar cross of 217–4 (41) hybrid hypohexaploid × 4n *P. axillaris* also yielded a large progeny, of which 16 plants were examined and all found to fall within the chromosome classes from 32 to 36 (table 4). The percentages of distribution are also included in table 4 and show that there is some factor acting to produce a higher range in the second cross. The reciprocal of the above crosses is found in 4n *P. axillaris* × hybrid hexaploid 224–5, which yielded only one plant, with the chromosome count of 35. Typical complements of the chromosome classes in the progenies of 6n × 4n crosses are seen in figures 18–24.

TABLE 3. *Chromosome class distribution of hexaploid progenies.*
The lower number in each generation is the percentage of the total.

| Chromosome classes | 40 | 41 | 42 | 43 | 44 | Total |
|---|---|---|---|---|---|---|
| C–1 generation | 6 | 3 | 16 | | | 25 |
| | 24 | 12 | 64 | | | |
| C–2 generation | 10 | 6 | 26 | 7 | 1 | 50 |
| | 20 | 12 | 52 | 14 | 2 | 75 |

## DISCUSSION

**Genetical Purity of the Primary Species.** While reference to and comparison with previous reports of experimental crosses within the genus *Petunia* will be made below, it should be emphasized that the crosses reported upon in this paper were made with two genetically pure species,

TABLE 4. *Chromosome class distribution in the progenies of hybrid hexaploid × 4n P. axillaris.*

Percentages of total plants falling within each chromosome class.

| Chromosome class | Seed parent 217–4 (6n) (41) | Per cent | Seed parent 217–12 (6n) | Per cent |
|---|---|---|---|---|
| 29 | 1 | 6.3 | | |
| 31 | 3 | 18.7 | | |
| 32 | 3 | 18.7 | 2 | 12.5 |
| 33 | 4 | 25.0 | 4 | 25.0 |
| 34 | 3 | 18.7 | 2 | 12.5 |
| 35 | 2 | 12.5 | 6 | 37.5 |
| 36 | 0 | 0.0 | 2 | 12.5 |

namely *P. axillaris* (Lam.) B.S.P. and *P. integrifolia* (Hook.) Schinz & Thellung. The population of *Petunia* based on these two species is unique in another respect in that the tetraploid female parent, 4n *P. axillaris*, is the first tetraploid form in the pure species *P. axillaris* (Stout & Chandler 1941; Stout 1945).

Ferguson and Ottley (1932) insist very strongly that in view of the frequent misuse in earlier investigations of the binomials *P. violacea* Lindl. and *P. nyctaginiflora* Juss., the gametic purity of the parents in any investigation should be determined at least for those characters which are to be studied in the succeeding generations. These authors refer more particularly to *P. violacea*, which they maintain with Steere (1932) is not found in a pure state in cultivation. Ferguson and Ottley (1932) cite evidence for the fact that the binomial *P. violacea* was used from 1836 onward as a "blanket tag" for small-flowered petunias grown in greenhouses and gardens and which were the hybrid varieties resulting from the crossing of the pure species *P. violacea* and *P. axillaris*. These pure species were imported to Europe from South America during the period 1823–1835.

The taxonomic history and specific characteristics of both *P. axillaris* and *P. violacea* are given by Ferguson and Ottley (1932). These binomials were retained by those authors after an analysis of the evidence for synonymy in these two species. The combinations *P. integrifolia* and *P. axillaris* have been used throughout this paper in view of recent detailed reinvestigation of synonymy in these two species by Stout (1947, written communication).

Previous reports of chromosome counts in the progenies of crosses in *Petunia* are found in the papers of Dermen (1931), Kostoff and Kendall (1931), Steere (1932), Matsuda (1935), Levan (1937), Stout and Chandler (1941, 1942) and Stout (1945). In the crosses reported before 1941, the only mention of a pure species used in a cross in *Petunia* is that of the diploid *P. axillaris* in the work of Steere (1932). There is a very notable difference between the diploid *P. axillaris* used by Stout and Chandler (1941, 1942) in

that the individual plants of Steere were self-fertile while those of Stout and Chandler were quite self-sterile. Stout (1947, personal communication) is of the opinion that the plants identified and figured as *P. axillaris* by Ferguson and Ottley (1932) and reported as 95 % self-fertile are not *P. axillaris*.

**A Fragment in a Diploid P. parodii.** This is apparently the first instance of a fragment reported in a pure diploid species of *Petunia*. Upcott (1936) cites the presence of such fragments in many plants and animals. A detailed study of such fragments occurring in diploids in *Tradescantia* has been made by Swanson (1943) but no certain conclusions could be drawn as to their origin. A sister plant of *P. parodii* did not contain the fragment (fig. 3).

**Tetraploid P. axillaris.** The somatic counts of the colchicine-induced tetraploid *P. axillaris* and the progeny of the selfed tetraploid are confirmations of the meiotic counts of Stout and Chandler (1941, 1942). It is to be noted that these authors reported the inactivation of the factors for incompatibility in the change from diploidy to tetraploidy (Stout & Chandler 1942; Stout 1945) and the hereditary transmission of induced tetraploidy and compatibility (Stout & Chandler 1942). All the determinations of these authors showed that there was general stability in the original tetraploid and its progeny inasmuch as all were exact tetraploids. Levan (1937) reported that of a progeny of 96 plants from a selfed 4n *P. violacea* 64 were exact tetraploids, 23 were in the hypotetraploid class with 27 chromosomes, 8 were hypertetraploid with 29 chromosomes, and one plant had 30 chromosomes. Matsuda (1935) reported that 29 of 31 plants in the progeny of a selfed tetraploid in *Petunia* were exact tetraploids. There is evidence given below that aneuploid gametes are formed in 4n *P. axillaris* but they do not apparently function in the selfing of the tetraploid. This would seem to be in agreement with the opinion of Müntzing (1931) and Levan (1942) that optimum viability exists when the egg and pollen have approximately the same chromosome number.

**Tetraploid-Diploid Crosses.** The segregates (22) from these crosses were all exact triploids with the exception of one plant which had the count of 20 and one with the count of 14. The cross 4n *P. axillaris* × 2n *P. axillaris* was reported earlier by Stout and Chandler (1942) as completely unsuccessful but Stout (personal communication) later found that the cross could be successful if it were between different genotypes.

There is a total of 129 counts for segregates from tetraploid-diploid crosses in *Petunia* in the combined reports of Kostoff and Kendall (1931), Steere (1932), Matsuda (1935), Levan (1937), and the present writer. The exact triploid class was found in 71.3 per cent of these 129 segregates. Mat-

suda (1935) is the only other investigator to report the exact reconstitution of the diploid from this type of cross. Diploid parthenogenesis and merogony are ascribed as the causes for the formation of these diploids. The tetraploids and near-tetraploids (hypotetraploids) reported by the above investigators are explained on the basis of fertilization and zygote formation from a diploid egg and diploid pollen grain (Matsuda 1935; Levan 1937). Hypertetraploids are due to diploid eggs with non-disjunctional chromosomes fertilized by diploid pollen (Matsuda 1935; Levan 1937).

The formation of triploids from the tetraploid-diploid cross is attributed to the union of a diploid egg and haploid pollen (Levan 1937). Experimental proof of such formation in *Crepis* is given by Navaschin (1929). It would be expected from the stability of the tetraploid *P. axillaris* that diploid eggs would be formed regularly. The zygotic viability of triploids with 2 genomes of *P. axillaris* and one of *P. integrifolia* is different from that of the triploid zygote containing 3 genomes of *P. axillaris* (Stout & Chandler 1942) and has been ascribed by Stout (personal communication) to the general rule that gametes containing similar factors for sterility, as in certain genotypes in the autotriploid zygote, are unviable.

**Triploid-Diploid Crosses.** The previous reports of the progenies of such crosses in *Petunia* are found in the reports of Matsuda (1935) and Levan (1937). Matsuda found that only 3 seeds out of 250 germinated and the plants on examination had the chromosome numbers 19, 20, and 21. This triploid (21) is the only report of such a segregate from a $3n \times 2n$ cross in *Petunia*. The expectation of obtaining a triploid from such a cross is very slight, as can be seen from the comparative data for such crosses in several genera by Levan (1942). Table 5 contains the previous reports of chromosomal counts in the progenies of triploid-diploid crosses in *Petunia* in conjunction with the findings of the present author. There is complete agreement between the findings of Levan and those presented here in respect to the number of classes found in the progeny. All the classes between 2n and $3n-1$ are found. The chromosome class with the greatest frequency is the trisomic (15), which includes 38.9 per cent of the segregates in Levan's material and 26.7 per cent in the author's material. If the classes 14, 15, and 16 are combined into one group, the value of this group in Levan's material is 85.5 per cent and 52.2 per cent in the material examined by the author. This heavy concentration in the diploid range is attributed to the non-random distribution in triploid meiosis that was noted early by Belling and Blakeslee (1922) and confirmed thereafter. In particular it may be noted that Satina and Blakeslee (1937b) found in triploid *Datura* in a study of the female gametophyte 30 times as many female gametes with $n+1$ chromosomes and 280 times as many haploid gametes as would be expected if ran-

dom distribution were taking place. These authors attribute the high number of diploid and near-diploid progeny to chromosomal elimination of lagging chromosomes in both meiotic divisions.

There is however some disagreement between the distribution of chromosome classes found in the progenies of 3n × 2n crosses as reported by Levan (1937) and by the present author. Using the scheme of Levan (1942), the author has divided the 2n–3n range into five groups: group 14, group 15–16, group 17–18, group 19–20 and group 21 (table 5). As can be seen, the two sets of distribution diverge sharply in group 19–20 but there is a general tendency to the higher chromosome classes in the author's material. The group 19–20 is represented by only 3.3 per cent in Levan's material but accounts for 24.4 per cent in the progenies reported by the author. This percentage (24.4) is higher than any listed for 3n × 2n crosses in several

TABLE 5. *Distribution of chromosome classes in progenies of 3n × 2n crosses in Petunia.*

| | Groups | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | | III | | IV | | V | | |
| Chromosome classes | 14(2n) | 15 | 16 | 17 | 18 | 19 | 20 | 21(3n) | | |
| Number of plants | | | | | | 1 | 1 | 1 | (Matsuda 1935) | |
| Number of plants | 18 | 35 | 24 | 5 | 5 | 2 | 1 | 0 | Levan 1937) | |
| Group % | 20.0 | 65.5 | | 11.1 | | 3.3 | | 0.0 | | |
| Number of plants | 4 | 12 | 9 | 4 | 5 | 3 | 8 | 0 | (Sullivan, present paper) | |
| Group % | 8.8 | 46.7 | | 20.0 | | 24.4 | | 0.0 | | |

genera in the review by Levan (1942). The highest figure given there for the comparable group (19–20) is 18.0 per cent for *Populus*, but this is considered to be a case of secondary polyploidy (Johnson 1942; Levan 1942). It may be that the triploid *Populus* resembles to some degree the hybrid triploid used in *Petunia* 3n × 2n crosses and reported upon here.

**Differences of Specific 3n × 2n Crosses.** The distribution of chromosome classes in the progenies of 3n × 2n crosses is different when the pollen parent *P. integrifolia* is substituted for *P. axillaris* (table 6). In addition there is considerable difference in the distribution depending on which 3n segregate is used as the seed parent in the cross (table 6). It seems that the 3n × 2n cross in which the pollen parent was *P. integrifolia* has a distribution that is much closer to that reported by Levan (1937) than the distribution of the 3n × 2n cross in which *P. axillaris* was the pollen parent. In the latter cross a most unusual distribution of the progeny tends to fall in the upper classes approaching the triploid. There is, moreover, no concentration in the trisomic class. The high frequency may have some explanation in that a

triploid, hybrid for one of 3 genomes (one genome of *P. integrifolia* and 2 of *P. axillaris*) might form more eggs that would contain more chromosomes homologous to those of *P. axillaris* and such eggs would function more frequently with haploid pollen of *P. axillaris* than with haploid pollen of *P. integrifolia*. It is most probable that the chromosomal complements of the various hybrid triploids are not identical and will produce a different distribution depending on the genetic make-up of the triploid. Although the number of plants involved in the particular 3n × 2n crosses is small, the percentages of distribution (table 6) seem to indicate that factors other than merely quantitative are operating to produce the distribution. The effects of

TABLE 6. *Distribution of progenies of individual 3n × 2n crosses in perigreed Petunia.*

| | Groups | | | | | Cross |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | |
| Chromosome classes | 14(2n) | 15 16 | 17 18 | 19 20 | 21(3n) | |
| Number of plants | 1 | 10 7 | 3 3 | 1 3 | 0 | 3n × 2n *P. integrifolia* |
| Group % | 3.6 | 60.7 | 21.4 | 14.3 | 0.0 | |
| Number of plants | 3 | 2 2 | 1 2 | 2 5 | 0 | 3n × 2n *P. axillaris* |
| Group % | 17.3 | 23.6 | 17.6 | 41.1 | 0.0 | |
| Number of plants | 0 | 4 6 | 2 0 | 0 0 | 0 | 3n × 2n *P. integrifolia* |
| Group % | 0.0 | 83.4 | 16.6 | 0.0 | 0.0 | |
| Number of plants | 1 | 6 1 | 1 3 | 1 3 | 0 | 3n × 2n *P. integrifolia* |
| Group % | 6.3 | 43.7 | 25.0 | 25.0 | 0.0 | |
| Number of plants | 0 | 1 1 | 1 2 | 1 4 | 0 | 3n × 2n *P. axillaris* |
| Group % | 0.0 | 20.0 | 30.0 | 50.0 | 0.0 | |
| Number of plants | 3 | 1 1 | 0 0 | 1 1 | 0 | 3n × 2n *P. axillaris* |
| Group % | 42.8 | 28.6 | 0.0 | 28.6 | 0.0 | |

specific factors for sterility and fertility are being analyzed by Dr. A. B. Stout. It may be noted that the inclusions of the various types of distribution of the individual crosses into a single general distribution produces a distribution that resembles that given by Levan (1942) for several genera but this masks the individual factors at work. The detailed analysis of each genotype in the cross is necessary if the true nature of such crosses is to be determined.

**Triploid-Triploid Crosses.** The reports of previous triploid crosses listed as the selfings of triploids, are found in Dermen (1931), Matsuda (1935), and Levan (1937). The triploid crosses reported on by the present author are different in that two presumably different hybrids are involved. The chromosome classes found in previous reports include the classes from the diploid 14 to 18 and from 26 to 29. It can be seen that the present report, giving the numbers 19, 20, 23, and 25, fills in the curve of distribution near

the triploid range that had been considered characteristically absent in triploid-triploid crosses in *Petunia* by Levan (1942). Levan compared the progenies of triploid-triploid crosses in 8 genera including *Petunia* and divided the range of the progenies into three groups comprising the ranges 2n to 3n – 1, 3n to 4n and 4n and upwards. Levan found that the progenies fell into two main groups, one with low basic haploid chromosome numbers and the other with high basic haploid numbers. The low basic haploid chromosome number group in which *Petunia* is found is subdivided according as the progeny of the triploids fell to the extent of 50 per cent or more into the lowest range, 2n to 3n – 1, or into the upper ranges to the same extent. The only member of the second group is *Populus* (Johnson 1942) which has a distribution that approaches the expected binomial distribution in the triploid progenies. Johnson (1942) and Levan (1942) suggest that this distribution is due to secondary polyploidy. The appearance in the author's material of chromosome classes in the triploid range would not argue against such a view but would stress that viability of the gametes is concerned with the formation of gametes with equal numbers of homologous chromosomes (Müntzing 1936). In this connection it may be noted that many authors have reported that the cross 2n × 3n invariably gives a high proportion of diploids and the progeny is limited to numbers close to the diploid. It would appear that equality of number in the gametes is an important factor in viability. Dermen (1931) reported that from such a cross there were reconstituted 39 diploids and 12 trisomics in 51 segregates, and Levan (1937) reported that the total progeny of such a cross consisted of 41 diploids. Apparently only haploid or disomic pollen functioned in these crosses. The work of Johnsn (1942) on *Populus* showed that the distribution of 2n × 3n and 3n × 2n was essentially the same, indicating that pollen grains are not more susceptible than embryo sacs to unbalanced chromosome numbers. There is every reason to believe that the pollen formed in triploids ranges from haploid to 2n – 1 from the observations of Dermen (1931) and from Satina and Blakeslee (1937a). It would seem that all classes of pollen function in the triploid crosses since nearly all classes between 2n and 4n are represented in the progenies. The numerical ratio of chromosomes in the egg and pollen grain is quite important as far as can be judged from the viable zygotes.

In the statistical analysis of triploid progenies of several genera, Levan (1942) found three types exemplified by *Beta* (Levan 1942), *Allium* (Levan 1936a, 1936b) and *Populus* (Johnson 1942). In *Beta*, the majority of the progeny fall between 2n and 3n and all theoretical expectations are well fulfilled. In *Allium*, however, most of the progeny approached the 4n class, while some of the lower classes are almost or entirely missing. In *Populus*, the entire range from 2n to 4n is well represented according to the

theoretical distribution expected, with maxima near 2n and 4n and a pronounced maximum at 3n. The analysis does not of course take into consideration the specific factors characteristic of each triploid used in the crosses. The very generally reported reduced fertility and frequent sterility of triploids and the reduced percentage of germination are probably dependent on the specific genotype of the individual triploid. Thus the counts reported for triploid-triploid crosses in *Petunia* indicate that there well may be a concentration at or near the 3n class depending on the specific genotypes employed in the cross.

The factors that influence the distribution of chromosome classes in triploid progenies away from the theoretically expected distribution are discussed at length by Levan (1942). The elimination of chromosomes appears to be a major factor in lowering the range of distribution. Upcott and Philip (1939) attribute much importance to the relative size of chromosomes and cells, in that small chromosomes will be more easily eliminated in large cells in which the distance to the poles from the equator is relatively long. Thus elimination would occur more easily in embryo sac mother-cells than in pollen mother-cells. Levan (1942) attributed little influence to these factors, nor did he find any significant relation between the functioning of pollen with the changed chromosomal relations of mother plant, embryo, and endosperm in polyploid crosses. Müntzing (1936) had suggested that such relations were of considerable importance.

In the analysis of triploid progenies of *Allium*, *Populus*, and *Beta*, Levan (1942) showed that less haploid pollen functions in zygotic production in $3n \times 3n$ than in $2n \times 3n$ crosses. Pollen grains with higher chromosome numbers are more successful in forming zygotes in the triploid than in the diploid crosses involving triploids. In the $3n \times 3n$ crosses 2n pollen grains are much more frequent than in the cross $3n \times 2n$. One explanation is the need of numerical equality of chromosomes in egg and pollen (Müntzing 1936). Levan concludes that zygotic selection is the cause of differences in the progenies of triploids in the three genera mentioned above. Thus also there appears to be a positive selection for near-diploid pollen in the progenies of triploids in *Petunia*, since nearly three-fourths of all such progenies belong to the near-tetraploid class, yet from the evidence of Satina and Blakeslee (1937a) all classes of pollen seem to be formed in such triploids.

**Triploid-Tetraploid Crosses.** The previous reports on this type of cross in *Petunia* are found in Dermen (1931), Matsuda (1935), and Levan (1937). The present author lists only two segregates from such a cross. These, combined with previous reports, bring the total of such segregates reported to 54. Of this total, 45 fall in the 27–28 class (tetraploid range). It would seem here that diploid pollen is formed and functions mainly with

eggs of near- or exactly diploid pollen. The range of previous segregates from such crosses extended from the class 24 to 35 (pentaploid) with classes 30, 32, 33, and 34 missing. Thus the plant with 20 chromosomes reported by the author as a segregate from the triploid-tetraploid cross is the lowest count for such a cross in *Petunia*. However, extreme variation in the progenies of similar crosses in other genera are reported. Müntzing (1937) reported in the genus *Dactylis* that the progeny included the classes from 3n + 1 to 8n. Müntzing noted the maximum present in the tetraploid range from such crosses. It may be suggested that the 20-chromosome plant reported by the present author originated from gametes with approximately equal numbers such as 10 and 10 or 11 and 9. The possibility of homology existing between such gametes seems relatively great in a cross between an autotetraploid and a triploid containing 2 genomes that are supposedly homologous with the genomes of the tetraploid.

**The Selfing of Hexaploids.** This is the first report of hexaploids in *Petunia*. The hexaploids were produced by colchicine treatment of a branch of a hybrid triploid (field no. 170–4) which contained 2 genomes of *P. axillaris* and 1 genome of *P. integrifolia*. These genomes are presumably doubled in the hexaploid. Twenty-five plants of the selfings of the colchicine-treated branch were examined. Exact hexaploids (42) were found in 16 plants, 6 plants had 40 chromosomes, and 3 plants were in the 41 class. Of the 50 plants examined from the selfings of derived hexaploids 26 were exact hexaploids, 10 were in the 40-chromosome class, 6 in the 41 class, 7 in the 43 class and 1 in the 44 class. It is evident that the colchicine-induced hexaploids and their derived progeny possess a mechanism for fairly stable sexual reproduction. The allopolploidy present in the hexaploids does not seem to disturb the stability. Müntzing (1936) considers that most natural polyploid races which flourish widely are allopolyploid to some degree. The attempt to identify specific chromosomes was unsuccessful, and since no meiotic studies have yet been made on these hexaploids, it is impossible at present to assign any cause for the stability of the hexaploids. However, it should be mentioned that the factors governing incompatibility in the diploid *P. axillaris* were inactivated in the change from the diploid to the tetraploid condition (Stout & Chandler 1941, 1942; Stout 1945). The stability of the sexual reproduction of the tetraploid is reflected in the hexaploid. It may then be that homology and numerical equality of chromosomes in egg and pollen are the main factors in this stability.

**Hexaploid-Tetraploid Crosses.** The distributions of chromosome classes in the progenies of hexaploid-tetraploid crosses are different according as a different hybrid hexaploid is used as the seed parent (table 4). It is of

course extremely improbable that the chromosome complements of any two hybrid hexaploids are identical. However, although the maximum in the distribution (table 4) in one cross is the chromosome class 33 and in the other it is the class 35 (pentaploid), there is still a pronounced concentration in the latter in the class 33. The differences in genotype alone do not seem to effect wide differences in the distributions in the progeny and in both crosses these same classes include most of the progeny. In the latter cross the entire distribution is shifted to the higher classes and includes two hyperpentaploids. In general it may be said that the eggs are formed with numbers close to the triploid and the pollen is formed with numbers close to the diploid. This might be expected in view of the stability of both tetraploids and hexaploids.

The sole plant from the reciprocal cross, $4n \times 6n$, was found to be an exact pentaploid. This is in agreement with the general observation that pollen grains with euploid numbers have a greater viability (Levan 1937). While the pollen may have been exactly triploid and the egg exactly diploid, slightly aneuploid gametes would probably have given the same result.

The high rate of constancy in the progenies of both selfed hexaploids and of the hexaploid-tetraploid crosses is perhaps due to the presence of 4 genomes of *P. axillaris* in both the hexaploid and tetraploid. Homology exists to a large extent between the tetraploid and the hexaploid and in this respect the hexaploid shows the characteristics of an autopolyploid. Müntzing and Müntzing (1942) in crosses between diploid and three different hexaploid biotypes of *Potentilla* obtained 28 pentaploids. These authors ascribe the pentaploid formation to the unreduced ovules of the diploid and the reduced (triploid) pollen of the hexaploids. These authors concluded that the result of a cross in *Potentilla* material is determined not only by the degree of sexuality present (apomixis may be present) but by the chromosome number and the genetic constitution of the male parent. Nordenskiöld (1937) found in *Phleum* that a cross of a triploid with a hexaploid gave mostly hexaploid plants. He ascribed this to the viability of the unreduced triploid gametes. In a hexaploid-tetraploid cross he obtained only three plants but all were pentaploids. He concluded that in the crosses in *Phleum* the four types used were closely related and the genomes more or less homologous. The difficulties encountered in crossing the various types were ascribed to quantitative differences in chromosome numbers rather than to any qualitative differences. It may also be said of the hexaploid crosses in *Petunia* that the viable zygotes are usually formed from eggs and pollen with approximately the same number of chromosomes. This number is euploid or only very slightly aneuploid. However, the loss or gain of one or two extra chromosomes does not affect the functioning of eggs and pollen in the higher polyploid forms. One hyperhexaploid (41) gave on selfing 11 plants. All the plants fell within

the chromosome classes 40 to 44 and 63.6 per cent were in the balanced hexaploid class (42). A similar range was found in the progeny of the balanced hexaploids.

## SUMMARY

1. The leaf smear technique combined with various pretreatments has been used to determine the somatic chromosome numbers of a pedigreed population of *Petunia* of Dr. A. B. Stout.

2. Confirmations of the diploid number of 14 for the pure species of *P. axillaris*, *P. integrifolia*, and *P. parodii* were made.

3. The chromosomes of the three diploid species are described as being within the range of 2.5 to 4.5 $\mu$ in length by 0.6 $\mu$ in width at metaphase. A SAT-chromosome is present in *P. axillaris*.

4. A chromosome fragment was reported in a pure diploid *P. parodii* and in a segregate from a hybrid 3n × 2n cross.

5. Confirmatory determinations of tetraploid forms of *P. axillaris* were made.

6. The progeny of 4n *P. axillaris* × 2n *P. integrifolia* consisted of 6 triploid and one hypotriploid with 20 chromosomes.

7. Fourteen plants from the cross 4n *P. axillaris* × 2n *P. axillaris* were determined as exact triploids and one as diploid (14).

8. All the chromosome classes between 2n and 3n − 1 were found in both the crosses hybrid 3n × 2n *P. integrifolia* and hybrid 3n × 2n *P. axillaris*. The distribution of the chromosome classes in the progeny of the latter cross tended to fall among the upper classes in the range 2n to 3n − 1. Comparison is made with similar previously reported crosses in *Petunia*.

9. The progeny of hybrid triploid-triploid crosses included 13 plants in the range of hypotriploid (19) to tetraploid (28). The chromosome classes 19, 20, and 23 are reported for the first time for such progenies.

10. Hybrid 3n × 4n *P. axillaris* gave only two plants, one with a count of 20 chromosomes and the other with 26 chromosomes.

11. Seventy-five plants, the progeny of the selfing of a colchicine-induced hexaploid hybrid and derived hexaploids, were all found to have chromosome numbers within the range from 40 to 44. Fifty-six per cent of the progeny were exact hexaploids.

12. The progenies of crosses of hybrid hexaploids × 4n *P. axilaris* all fell within the chromosome classes from 29 to 36 but the class 30 was not represented. Twenty-five per cent of the progenies were exact pentaploids. Individual crosses showed minor differences in distribution due perhaps to the different chromosome complements of the seed parent.

13. The reciprocal cross 4n *P. axillaris* × hybrid hexaploid gave only one plant with a count of 35 (pentaploid).

14. A pretreatment using podophyllin and chloral hydrate either in sequence or in combination was reported as a satisfactory substitute for colchicine for the study of the morphology of chromosomes.

15. A comparison with previous reports of similar crosses in *Petunia* was made with a discussion concerning the possible factors operating to produce chromosomal variation in the progenies.

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# A SURVEY OF 500 BASIDIOMYCETES FOR ANTIBACTERIAL ACTIVITY

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A previous publication from this laboratory (7) presented the results of a survey for antibacterial activity of more than 400 fungi, including over 300 Basidiomycetes, against *Staphylococcus aureus* and *Escherichia coli*. The present paper is a report of a survey of the antibacterial activity of 508 additional isolations of Basidiomycetes against the same indicator organisms. The procedures used were substantially those of the earlier investigation; differences are noted later in this paper.

Recent studies of the Basidiomycetes suggest that they are a promising source of antibiotic substances. Wilkins and Harris (12) tested extracts of the sporophores of 722 species in 96 genera of the larger Basidiomycetes for antibacterial activity against *Staph. aureus*, *E. coli*, and *"Pseudomonas pyocyanea."* They used the "hole" method (10) and a fungus was given a positive rating if the zone of inhibition of the bacterium was at least 2 mm. About 20 per cent were positive against *Staph. aureus* alone or *Staph. aureus* and *E. coli*. None was active on *E. coli* alone. Five species were active with *"P. pyocyanea."*

Wilkins (8) tested 56 isolations of Basidiomycetes grown on liquid and agar media. The liquids were tested by the "hole" method and the cultures grown on agar were tested by the "strip" method (11). Liquids active against *Staph. aureus* were obtained with 28 of the cultures; 11 were active against *Staph. aureus* and *E. coli;* 3 were active against *E. coli* and not against *Staph. aureus*.

Wilkins (9) reported the results on 100 additional Basidiomycetes grown on agar and in liquid, and tested by the "strip" and "hole" methods respectively against *Staph. aureus* and *E. coli*. About 10 per cent were strongly positive, 20 per cent were weakly positive and the rest were negative.

Robbins and his associates (7) in a survey of over 400 fungi, including over 300 Basidiomycetes, found that about 50 per cent displayed antibacterial activity. One hundred nineteen fungi produced zones of inhibition of more than 10 mm. for *Staph. aureus* by the streak method. None was active against *E. coli* alone. Two fungi were found to produce culture fluids active against *Staph. aureus* at a dilution of 1 to 1024.

Hollande (4) reported that clitocybine, an antibiotic substance from *Clitocybe gigantea* var. *candida* is active against several bacteria including *Mycobacterium tuberculosis*, *Bacillus typhosus*, and *Brucella abortus*. Posi-

tive therapeutic results were reported on guinea pigs infected with tuberculosis.

Mathieson (6) tested the extracts of the sporophores of 230 species of Australian Basidiomycetes for antibacterial activity. He used the "hole" method of Wilkins and Harris. An inhibition zone of 2 mm. was considered positive. One hundred seventy gave negative results; 39 were active against *Staph. aureus* alone; 20 were active against *Staph. aureus* and *E. coli;* and one was active against *E. coli* alone.

In an investigation of over 200 types of toadstools and mushrooms, Atkinson (1) found only five with activity against *Staph. aureus.* Two organisms, *Cortinarius rotundisporus* and *Psalliota xanthoderma,* were active against *Staph. aureus* and *"B. typhosum."* Further work on extracts of the sporophores from these two species indicated that both fungi were active against a wide range of bacteria. The activity of both extracts was unaffected by the presence of 10 per cent serum, and the toxicity to animals was low.

Bose (2) reported that polyporin, the filtrate of *Polystictus sanguineus* grown in a supplemented Czapek-Dox medium, is active against *Staph. aureus, Streptococcus pyogenes, Streptococcus viridans, B. typhosus, B. paratyphosus* A and B, *E. coli, Vibrio cholerae,* and *B. flexner.* Polyporin was stated to be non-toxic to animals.

## METHODS AND MATERIALS

Five hundred and eight isolations of Basidiomycetes were tested for antibacterial activity against *Staph. aureus* and *E. coli.* The streak method was used as a preliminary screening test. The fungi found to be most effective in inhibiting the growth of one or both indicator bacteria and which were not strong acid producers were tested further for activity by the agar disc method. The fungi displaying marked antibacterial activity by both the streak and disc methods were then cultivated in liquid media and the antibacterial activity of the culture fluids was determined by serial dilution tests. Several related problems were investigated as described later.

The fungi were obtained from various sources.[1] Five isolations came from the Centraalbureau voor Schimmelcultures, Baarn, Holland (CBS), 49 from Dr. Dow V. Baxter, University of Michigan (B), 159 from Dr. José Emilio Santos Pinto Lopes, Instituto Botanico, Lisbon, Portugal (L), and 295 from Dr. Mildred K. Nobles, Central Experimental Farm, Ottawa, Canada (N).[2] These cultures included 64 genera and 352 species. Twelve isolations were unidentified as to species.

[1] This investigation was supported in part by grants from the Winthrop Chemical Company and the Commonwealth Fund.

[2] The letters in parentheses are used in this report to indicate the source of the cultures. Hereafter, these letters will be used before the stock numbers of the cultures.

The assistance of Dr. Baxter, Dr. Lopes, and Dr. Nobles in supplying the fungi tested in this survey is appreciated. It would not have been possible to have completed the work reported here without the cultures so freely and generously supplied.

The author wishes to thank Dr. G. W. Rake, Squibb Institute for Medical Research, for the cultures of *Staphylococcus aureus* (Heatley strain) and *Mycobacterium phlei*, Dr. Selman A. Waksman, New Jersey Agricultural Experiment Station, for the culture of *Escherichia coli*, and Dr. H. Boyd Woodruff, Merck and Co., Inc., for the culture of *Pseudomonas aeruginosa*.

Details on the methods and media are included in the sections which follow.

## EXPERIMENTAL WORK

**Survey by the Streak Method.** The isolations of fungi used in this survey were first tested for antibacterial activity by the streak method. Two media were found to be generally suitable for the growth of the fungi and of the indicator bacteria. These were a modified Czapek-Dox medium prepared with 5 g. corn-steep solids per liter[3] and a thiamine peptone agar.[4] Each fungus was grown on 20 ml. of each medium in Petri dishes $15 \times 100$ mm. at 25° C. After several days, depending upon the rapidity of growth of the fungus, the plates were streaked with 6–8 hour AC[5] broth cultures of *Staph. aureus* and *E. coli* starting at the edge of the fungus colony and extending to the periphery of the dish. The plates were incubated overnight at $37 \pm 2°$ C and when inhibition of the indicator bacteria was observed measurements were taken in millimeters from the edge of the fungus colony to the growth of the bacteria. Alkacid indicator paper (Fisher Scientific Co.) was used to test the acidity next to the fungus colony. Each isolation was usually tested for antibacterial activity at two different ages for each medium. The age of the colony, when tested, ranged from 4 to 28 days with two exceptions. The second tests on *Polyporus amorphus* (N16597) and *Omphalia campanella* (N11761) were made after 38 days because of the very slow growth of these organisms on the media used.

**Results with the Streak Method.** Two hundred and thirty-three fungi showed no activity against *Staph. aureus* or *E. coli* on both media. These were:[6]

[3] The solution contained per liter of distilled water 3 g. $NaNO_3$, 1 g. $KH_2PO_4$, 0.5 g. KCl, 0.5 g. $MgSO_4 \cdot 7H_2O$, 40 g. dextrose and 15 g. Difco agar. The corn-steep solids were in Special Nutrient #14 from A. E. Staley Co. The pH was 5.7 after autoclaving.

[4] The thiamine peptone agar contained per liter 1.5 g. $KH_2PO_4$, 0.5 g. $MgSO_4 \cdot 7H_2O$, 1 g. neopeptone, 10 g. dextrose, 15 g. Difco agar and 600 mμmoles of thiamine. Mineral supplements in p.p.m. were added as follows: 0.005 B, 0.02 Cu, 0.10 Fe, 0.01 Ga, 0.01 Mn, 0.01 Mo and 0.09 Zn. The pH was 5.5 after autoclaving.

[5] The AC broth contained per liter 20 g. proteose peptone #3, 3 g. yeast extract, 3 g. meat extract, 3 g. malt extract, 5 g. dextrose. The pH was 6.8 after autoclaving.

[6] An asterisk signifies that a disc test was made.

*Agrocybe semiorbicularis* (L92)
*Armillaria mellea* (L255)
*Auricularia auricula* (N16631)
*Clitocybe geotropa* (L179)
*C. illudens* (N10018)
*C. tabescens* (L210, NF2337)
*Collybia fusipes* (L204)
*C. radicata* (NF3461)
*C. velutipes* (NF1598)
*Conocybe pubescens* (L57)
*C. lateritia* (L464)
*Coprinus boudierii* (L60, L60.6, L268)
*C. coopertus* (L218)
*C. curtus* (L1, L141, L380)
*C. domesticus* (L2, L166, L379)
*C. ephemerus* (L121)
*C. fimetarius* (L414)
*C. fimetarius* (?) (L181)
*C. friesii* (L142)
*C. hiascens* (L200, L285, L378)
*C. impatiens* (L462)
*C. lagopus* (L4)
*C. micaceus* (NF290, L350, L364)
*C. radians* (L17, L86, L87)
*C. radiatus* (L374)
*C. stercorarius* (L91)
*C.* sp. (L375, L397, L397.4, L397.5)
*Corticium alutaceum* (NF6328)
*C. coeruleum* (NF2936)
*C. galactinum* (N16568)
*Crucibulum vulgare* (L438)
*Cyathus striatus* (L437)
*Daedalea ambigua* (NF 2017)
*D. biennis* (NF7302)
*D. confragosa* (N9210)
*D. gibbosa* (NF7309)
*Deconica crobula* (L107)
*D. inquilina* (L54.27)
*Drosophila appendiculata* (L25.8, L108)
*D. badia* (L242, L242.14, L341)
*D. candollcana* (L202)
*D. exalbicans* (L127, L127.4)
*D. fatua* (CBS22)
*D. gracilis* (L460)
*D. lactea* (L459)
*D. pygmaea* (CBS24, L391.6)
*D. spadicophylla* (L448)
*D.* sp. (L376, L444)
*Favolus canadensis* (N11794)
*Flammula carbonaria* (L66)
*F. gummosa* (L161)
*F. ochroleuca* (L315)
*Fomes calkinsii* (NF1819)
*F. conchatus* (NF10159)
*F. connatus* (N10254)
*F. everhartii* (NF3580)
*F. extensus* (NF7325)
*F. fomentarius* (N11264)
*F. fraxineus* (NF7519)
*F. geotropus* (NF2023)
*F. igniarius* (N8207)
*F. igniarius laevigatus* (NF1956)
*F. lobatus* (NF8008)
*F. marmoratus* (NF2024)
*F. nigricans* (B707)
*F. nigrolimitatus* (N10085A)
*F. noxius* (N9229)
*F. rimosus* (NF2164)
*F. robustus* (NF1656)
*F. robustus tsuginus* (N8219)
*F. scutellatus* (NF3444)
*Fomitiporia dryophila* (B713, B714)
*F. earleae* (B715)
*F. flavomarginata* (B716)
*Galera tibicystis* (L94)
*Grandinia granulosa* (N16567)
*Lentinus lepideus* (N11770)
*L. tigrinus* (L38.1, L38.3, L38.11)
*L. tigrinus* (?) (NF2030)
*Lenzites betulina* (N10199)
*L. flaccida* (NF7304)
*L. repanda* (N9231)
*L. thermophila* (NF1682)
*L. tricolor* (NF7327)
*Lycoperdon gemmatum* (NF2944)
*Melanoleuca cognata* (L63)
*Merulius corium* (L321)
*Mucidula radicata* (L111)
**Mycena atroalba* (L359)
*Panus rudis* (NF1927)
*Peniophora corticalis* (L360)
*P. cremea* (NF2946)
*P. gigantea* (NF7329)
*P. populnea* (N10740)
*Phlebia mellea* (N16566, N16569, N16626, N16642)
*Pholiota adiposa* (N8457)
*P. aurivella* (N8485)
*P. mutabilis* (L208)
*P. spectabilis* (NF6627)
*P. squarrosa* (L313)
*Pholiotina togularis* (L465)
*Pleurotus ostreatus* (N9220, L361)
*P. sapidus* (NF1610)
*P. serotinus* (NF2174)
*Polyporus abietinus* (N9517)
*P. adustus* (N11810)
**P. alboluteus* (N8263, N16598)
*P. albosordescens* (NF7330)
*P. anceps* (NF3372, N16565, N16565K)
*P. arcularius* (NF3768)
*P. benzoinus* (NF6606, B725)

*P. borealis* (N16610)
*P. brumalis* (N11804)
*P. cinnabarinus* (NF1609)
*P. circinatus* (N10910)
*P. circinatus dualis* (N11617)
*P. conchifer* (N10210)
*P. croceus* (NF2178)
*P. cuticularis* (N10194)
*P. distortus* (NF8002)
*P. dryadeus* (N9238)
*P. dryophilus* (NF2179)
*P. elegans* (N11785)
*P. fibrillosus* (N10909)
*P. frondosus* (N10235)
*P. fumosus* (N11813)
**P. galactinus* (NF3494)
*P. glomeratus* (N11254)
*P. graveolens* (NF2954)
*P. hirsutus* (N11805)
*P. hispidus* (NF2038)
*P. imberbis* (N9232)
*P. lucidus* (N10222)
*P. pargamenus* (NF8016)
*P. picipes* (NF1352)
*P. pubescens* (N11616, N16660)
*P. resinosus* (N11611*, N16625, N16659)
*P. robiniophilus* (NF2185)
*P. spumeus mongolicus* (NF2288)
*P. squamosus* (N10789)
*P. tamaricis* (NF7337)
*P. tenuis* (NF2048)
*P. tuberaster* (NF7997b)
**P. tulipiferus* (NF8049)
*P. umbellatus* (N9263)
*P. velutinus* (N10238)
*P. versicolor* (N11812)
*P. zonatus* (NF7389)
*Poria albipellucida* (N11692, B737)
*P. albobrunnea* (B738)
*P. ambigua* (N8448)
*P. aurea* (N16609)
*P. cinerascens* (N9267)
*P. crustulina* (N16600)
*P. ferruginosa* (N11253)
*P. floridana* (B767)
*P. hymenocystis* (NF7315)
*P. laevigata* (B770, B771)
*P. mollusca* (B777)
*P. obliqua* (NF7403)
*P. prunicola* (NF1409)
*P. pulchella* (B784)
*P. punctata* (NF1563)
*P. rufa* (N16560, N16564, N16615, N16616, B786)
*P. semitincta* (B787)
*P. sericeo-mollis* (NF8017)
*P. subfusco-flavida* (B795)
*P. tacamahacae* (B796)
*P. tsugina* (B797)
*P. undata* (B799)
*P. xantha crassa* (B808)
*P.* sp. (N16646, B789)
*Pseudocoprinus disseminatus* (L16)
*Radulum casearium* (N16599)
*Schizophyllum commune* (L443, N11795)
*Stereum abietinum* (N16603)
*S. fasciatum* (NF7341)
*S. gausapatum* (N9246)
*S. rugosum* (N9245)
*S. sanguinolentum* (N9504)
*S. spadiceum* (N8644)
*Stropharia merdaris* (L23, L223)
*Trametes gibbosa* (L319)
*T. hispida* (NF6005)
*T. robiniophila* (NF3773)
**T. serpens* (B)
*T. suaveolens* (NF7654)
*Tricholoma ambustum* (L269)

Zones of inhibition less than 5 mm. against *Staph. aureus* were obtained with the following 26 isolations:

*Armillaria mellea* (NF2508)
*Clitocybe phyllophila* (L463)
*Clitopilus abortivus* (NF3517B6)
*Coprinus extinctorius* (L203)
*C. hiascens* (L200.3)
*Drosophila appendiculata* (L20, L84)
*D. caudata* (L458)
*D. marcescibilis* (CBS26)
*D. prona* (L332)
*Fomes fulvus* (NF2283)
**Lenzites saepiaria* (N7528)
*Panaeolus retirugis* (L18)
*Peniophora quercina* (NF8089)
**Pluteus nanus* (L225)
*Poria cupora* (N11252, B753)
*P. mutans tenuis* (B779)
*P. viridiscula* (B801)
**P. weirii* (N9422)
**P. xantha* (N9315)
*P. xantha* f. *crassa* (B811)
*Stereum abietinum* (N16604)
*S. sulcatum* (N16619)
*Trametes mollis* (NF2351)
*T. tenuis* (N9265)

Most of these fungi showed no inhibition of *E. coli*. *Armillaria mellea* (NF2508), *Clitocybe phyllophila* (L463), *Lenzites saepiaria* (N7528), and *Peniophora quercina* (NF8089) produced zones of inhibition less than 5 mm. on *E. coli*. Zones of inhibition slightly more than 5 mm. were produced with *Pluteus nanus* (L225) and *Stereum sulcatum* (N16619).

Zones of inhibition for *Staph. aureus* ranging from 5 to 10 mm. as a maximum were produced by 46 of the isolations tested. There were:

*Corticium effuscatum* (NF5322)
*Daedalea flavida* (NF7301)
*Drosophila candolleana* (L202.1, L202.5)
*D. sarcocephala* (L26)
*D.* sp. (L382)
*Fomes ellisianus* (NF7520)
*F. igniarius robustus* (N9228)
*F. nigrolimitatus* (B708)
**F. pini* (N16632)
*F. subroseus* (NF1639)
**Hydnum abietis* (N16602)
**Hymenochaete tabacina* (N9252)
*Lentinus kauffmanii* (N11660)
**Lenzites trabea* (NF3823)
*Merulius confluens* (NF2945)
**Panaeolina foenisecii* (L10)
*Panaeolus campanulatus* (L466)
*P. papilionaceus* (L387.8, L468)
*Pholiota destruens* (NF1566)
*P. mutabilis* (L208.5)
*P. sphaleromorpha* (L132)
*Polyporus berkeleyi* (NF2952)
*P. betulinus* (N16656)
*P. borealis* (NF3581)
*P. caesius* (NF6891)
*P. gilvus* (NF1965)
**P. hydnoides* (NF2039)
**P. oregonensis* (N8240)
**P. sulphureus* (NF3474)
**P. tsugae* (N9346)
*Poria alaskana* (B736)
*P. crustulina* (B751)
*P. cupora* (B752)
**P. ferrea* (N9887)
*P. medullapanis* (B775)
*P. nigrescens* (N16605)
*P. rufa* (N16617)
*P. weirii* (N16607)
*P.* sp. (B790)
**Stereum pini* (NF2963)
**S. subpileatum* (NF2056)
**Trametes isabellina* (NF7338)
**T. serpens* (B832)
*T. variiformis* (N16572)

The antibacterial activity of most of these fungi as indicated by the streak test was limited to *Staph. aureus*. With *Fomes ellisianus* (NF7520), *Lenzites trabea* (NF3823), *Pholiota sphaleromorpha* (L132), *Stereum pini* (NF2963), and *S. subpileatum* (NF2056) there was an inhibition zone of less than 5 mm. on *E coli*. The zone of inhibition for *E coli* ranged from 5–10 mm. as a maximum with *Fomes pini* (N16632), *Hymenochaete tabacina* (N9252), *Panaeolina foenisecii* (L10), *Polyporus berkeleyi* (NF2952), *P. betulinus* (N16656), *Poria ferrea* (N9887), *Trametes isabellina* (NF7338), and *T. variiformis* (N16572). One fungus in this group, *Polyporus caesius* (NF6891), produced a larger inhibition zone with *E. coli* than with *Staph. aureus*.

Inhibition zones of more than 10 mm. for *Staph. aureus* were obtained with 203 of the 508 isolations on one or both media used in the survey. The results for these fungi are summarized in table 1. For each of these fungi the largest zones of inhibition against *Staph. aureus* and *E. coli* on both the corn-steep and the thiamine peptone agars are given. The A in the last column designates that the fungus produced a considerable amount of acid.

TABLE 1. *Antibacterial activity of fungi tested by streak method.* The maximum inhibition zones in mm. are given against *Staph. aureus* and *E. coli* for the fungi grown on the corn-steep or the thiamine peptone agar. "A" indicates that a considerable amount of acid was produced by the fungus. Those marked with an asterisk were also tested by the agar disc method.

| Organism | Corn steep agar | | Thiamine peptone agar | | Acidity |
|---|---|---|---|---|---|
| | *Staph. aureus* | *E. coli* | *Staph. aureus* | *E. coli* | |
| **Agrocybe dura* (L386) | 30 | 30 | 60 | 51 | |
| **A. dura* (L386.10) | 50 | 50 | 50 | 50 | |
| **A. dura* (L386.14) | 50 | 50 | 55 | 55 | |
| **A. semiorbicularis* (L92.1) | 16 | 10 | 20 | 22 | |
| **A. semiorbicularis* (L92.6) | 23 | 11 | 30 | 9 | |
| **Alnicola escharoides* (L205) | 35 | 0 | 18 | 0 | |
| *A. melinoides* (L206) | 10 | 0 | 15 | 0 | |
| **Claudopus nidulans* (NF1996) | 21 | 3 | 22 | 8 | |
| **C. nidulans* (N16627) | 25 | 4 | 45 | 8 | |
| **Clitocybe ditopa* (L357) | 20 | 12 | 30 | 17 | |
| **C. obsoleta* (L358) | 70 | 13 | 75 | 3 | |
| **C. robusta* (NF2932) | 16 | 0 | 35 | 0 | |
| **Collybia dryophila* (L62) | 16 | 14 | 40 | 40 | A |
| **C. dryophila* (L72) | 29 | 29 | 45 | 38 | A |
| **C. dryophila* (L109) | 18 | 18 | 29 | 29 | |
| **C. erythropus* (L30) | 31 | 25 | 40 | 32 | |
| **Coniophora puteana* (N9779) | 11 | 5 | 6 | 6 | |
| **C.* sp. (N16648) | 3 | 4 | 14 | 16 | |
| **Coprinus auricomus* (L58) | 20 | 0 | 23 | 0 | |
| **C. comatus* (NF2935) | 2 | 2 | 25 | 23 | |
| **C. picaceus* (L14) | 32 | 0 | 30 | 0 | |
| **C. plicatilis* (L461) | 25 | 0 | 40 | 0 | |
| **C. similis* (L3) | 8 | 8 | 15 | 11 | |
| **Corticium portentosum* (N9283) | 30 | 0 | 4 | 0 | |
| **Crepidotus mollis* (L73) | 0 | 0 | 15 | 20 | |
| **Daedalea quercina* (NF2278) | 10 | 10 | 23 | 18 | A |
| **D. unicolor* (N10202) | 22 | 0 | 7 | 0 | |
| **Deconica inquilina* (L54a) | 20 | 0 | 0 | 0 | |
| **D. inquilina* (L54b) | 17 | 0 | 10 | 0 | |
| **Drosophila appendiculata* (L25) | 45 | 0 | 30 | 0 | |
| **D. caudata* (L277) | 30 | 0 | 8 | 0 | |
| **D. caudata* (L277.7) | 27 | 0 | 37 | 0 | |
| **D. caudata* (L278) | 70 | 0 | 60 | 0 | |
| **D. exalbicans* (L450) | 59 | 0 | 65 | 30 | |
| **D. fibrillosa* (CBS23) | 70 | 0 | 50 | 0 | |
| **D. fibrillosa* (L15) | 42 | 0 | 60 | 0 | |
| **D. fibrillosa* (L152) | 52 | 0 | 57 | 0 | |
| **D. gracilis* (L171.4) | 35 | 0 | 35 | 0 | |
| **D. hydrophila* (L474) | 0 | 0 | 25 | 0 | |
| **D. orbitarum* (L445) | 5 | 0 | 12 | 0 | |
| **D. semivestita* (L281) | 50 | 25 | 62 | 33 | |
| **D. subatrata* (CBS25) | 51 | 24 | 60 | 42 | |
| **D. subatrata* (L43.1) | 60 | 42 | 69 | 27 | |
| **D. subatrata* (L122) | 55 | 24 | 50 | 50 | |
| **D. subatrata* (L284) | 53 | 29 | 63 | 30 | |
| **D. subatrata* (L284.3) | 56 | 34 | 65 | 35 | |
| **D. subatrata* (L284.6) | 60 | 20 | 71 | 31 | |
| **D. subatrata* (L449) | 46 | 9 | 60 | 31 | |
| **D. trepida* (L247) | 33 | 0 | 24 | 0 | |

TABLE 1 (*Continued*)

| Organism | Corn steep agar | | Thiamine peptone agar | | Acidity |
|---|---|---|---|---|---|
| | *Staph. aureus* | *E. coli* | *Staph. aureus* | *E. coli* | |
| **D. trepida* (L247.3) | 60 | 0 | 29 | 7 | |
| **D. vernalis* (L367) | 48 | 0 | 60 | 30 | |
| **D.* sp. (L395.2) | 27 | 0 | 0 | 0 | |
| **Echinodontium tinctorium* (N8740) | 37 | 0 | 22 | 0 | |
| **Flammula sapinea* (L31) | 16 | 0 | 21 | 0 | |
| **Fomes annosus* (N8273) | 33 | 17 | 55 | 21 | |
| **F. applanatus* (N9295) | 0 | 0 | 17 | 15 | |
| **F. fraxinophilus* (NF1931) | 0 | 0 | 19 | 18 | |
| **F. juniperinus* (NF3765) | 7 | 5 | 48 | 46 | |
| **F. meliae* (NF2025) | 27 | 12 | 30 | 23 | A |
| **F. nigrolimitatus* (N16651) | 0 | 0 | 12 | 6 | |
| **F. nigrolimitatus* (N16652) | 0 | 0 | 20 | 13 | |
| **F. officinalis* (N9503) | 65 | 1 | 55 | 30 | A |
| **F. pini* (NF2162) | 19 | 18 | 25 | 21 | A |
| **F. pini* (N16612) | 60 | 41 | 48 | 42 | A |
| **F. pini* (N16635) | 18 | 18 | 18 | 15 | A |
| **F. pinicola* (N8568A) | 15 | 12 | 18 | 17 | A |
| **F. ribis* (NF2284) | 14 | 10 | 10 | 7 | |
| **F. roseus* (NF5565) | 15 | 10 | 18 | 13 | |
| **F. spadiceus* (N8102) | 0 | 0 | 30 | 0 | |
| **Hydnum abietinum* (N16601) | 3 | 0 | 17 | 0 | |
| **Hypholoma sublateritium* (NF2168) | 6 | 0 | 11 | 0 | |
| *Hypochnus centrifugus* (NF6896) | 17 | 12 | 25 | 17 | A |
| **Irpex cinnamomeus* (NF2169) | 40 | 40 | 35 | 20 | A |
| **I. mollis* (NF3047) | 6 | 0 | 15 | 0 | |
| **Lentinus cochleatus* (L130) | 15 | 0 | 22 | 0 | |
| **L. degener* (L45) | 5 | 5 | 24 | 20 | |
| **L. tigrinus* (L38.2) | 55 | 0 | 25 | 35 | |
| **Lenzites bicolor* (NF7326) | 11 | 4 | 10 | 0 | |
| **Lepiota naucina* (NF2170) | 24 | 20 | 25 | 15 | |
| **Marasmius candidus* (L149) | 7 | 0 | 19 | 0 | |
| **Merulius corium* (L273) | 12 | 12 | 0 | 0 | |
| **M. lachrymans* (NF3526) | 35 | 28 | 65 | 55 | A |
| **Mycena capillaris* (L291) | 20 | 0 | 21 | 0 | |
| **Omphalia campanella* (N11761) | 0 | 0 | 38 | 0 | |
| **O. flavida* (NF5711) | 15 | 15 | 9 | 8 | |
| **Panaeolus acuminatus* (L467) | 12 | 7 | 5 | 0 | |
| **P. campanulatus* (L371) | 0 | 0 | 15 | 0 | |
| **P. papilionaceus* (L387.2) | 15 | 0 | 0 | 0 | |
| **P. papilionaceus* (L387.3) | 55 | 3 | 30 | 0 | |
| **P. papilionaceus* (L387.9) | 60 | 15 | 50 | 6 | |
| **P. sub-balteatus* (L330) | 55 | 0 | 15 | 0 | |
| **P. sub-balteatus* (L482) | 15 | 0 | 2 | 0 | |
| **Panellus stypticus* (L369) | 15 | 16 | 39 | 26 | A |
| **Paxillus panuoides* (NF3007) | 22 | 12 | 27 | 8 | |
| **Peniophora incarnata* (NF8090) | 70 | 70 | 26 | 5 | |
| **P. pubera* (NF3456) | 0 | 0 | 12 | 0 | |
| **Pholiota lucifera* (NF673) | 15 | 4 | 24 | 13 | |
| **P. sphaleromorpha* (L132.2) | 11 | 8 | 28 | 28 | |
| **P. sphaleromorpha* (L132.3) | 11 | 10 | 4 | 7 | |
| **P. sphaleromorpha* (L132.6) | 11 | 10 | 15 | 14 | |
| **Pleurotus griseus* (NF2948) | 23 | 0 | 13 | 0 | |
| **P. ulmarius* (NF2950) | 55 | 0 | 25 | 0 | |

TABLE 1 (*Continued*)

| Organism | Corn steep agar | | Thiamine peptone agar | | Acidity |
|---|---|---|---|---|---|
| | *Staph. aureus* | *E. coli* | *Staph. aureus* | *E. coli* | |
| *Polyporus abietinus* (N16634) | 0 | 0 | 11 | 5 | |
| **P. albellus* (N11608) | 18 | 0 | 22 | 0 | |
| **P. amorphus* (N8552) | 42 | 7 | 32 | 10 | |
| **P. amorphus* (N16597) | 68 | 0 | 45 | 0 | |
| **P. amorphus* (N16613) | 38 | 0 | 35 | 7 | |
| **P. balsameus* (N8407) | 0 | 0 | 12 | 9 | |
| **P. betulinus* (N11610) | 0 | 0 | 20 | 18 | |
| **P. caesius* (NF6891) | 7 | 0 | 10 | 13 | |
| **P. compactus* (NF8004) | 10 | 0 | 14 | 0 | |
| **P. dichrous* (N11609) | 9 | 5 | 18 | 17 | |
| **P. dryophilus vulpinus* (NF4394) | 0 | 0 | 30 | 26 | A |
| *P. durus* (N9234) | 6 | 6 | 20 | 20 | A |
| **P. fragilis* (N10227) | 12 | 3 | 18 | 3 | |
| **P. giganteus* (NF3769) | 31 | 31 | 6 | 0 | A |
| **P. guttulatus* (N11603) | 0 | 0 | 22 | 15 | |
| *P. helveolus* (NF7334) | 10 | 7 | 26 | 25 | A |
| **P. immitis* (?) (N10215) | 37 | 37 | 57 | 47 | A |
| *P. intybaceus* (NF7311) | 0 | 0 | 15 | 14 | |
| **P. mollis* (NF6827) | 21 | 15 | 45 | 45 | |
| **P. montanus* (N8449) | 0 | 0 | 15 | 5 | |
| **P. nidulans* (N9312) | 0 | 0 | 25 | 0 | |
| **P. obtusus* (NF6798) | 20 | 0 | 25 | 0 | |
| **P. osseus* (N11701) | 0 | 0 | 25 | 16 | |
| *P. palustris* (N10618) | 12 | 11 | 22 | 16 | A |
| **P. radiatus* (N10229) | 17 | 0 | 24 | 0 | |
| **P. schweinitzii* (NF7448) | 5 | 4 | 25 | 0 | |
| **P. semipileatus* (NF3499) | 40 | 9 | 30 | 12 | |
| **P. spraguei* (NF2182) | 0 | 0 | 16 | 12 | |
| *P. stypticus* (N9223) | 41 | 39 | 59 | 51 | A |
| **P. subcartilagineus* (N9417) | 11 | 13 | 49 | 50 | A |
| **P. tephroleucus* (?) (N10902) | 30 | 20 | 21 | 18 | |
| *P. vaporarius* (NF1287) | 20 | 14 | 35 | 35 | A |
| *P. vaporarius* (B735) | 20 | 20 | 35 | 35 | A |
| *P. vulpinus* (NF2279) | 20 | 18 | 8 | 0 | A |
| **P. zonalis* (NF2049) | 41 | 0 | 5 | 0 | |
| **Poria albobrunnea* (N16574) | 14 | 9 | 10 | 10 | |
| **P. candidissima* (N11613) | 5 | 0 | 30 | 0 | |
| **P. carbonica* (N8247) | 0 | 0 | 25 | 23 | |
| **P. colorea* (N11791) | 26 | 0 | 8 | 0 | |
| **P. ferrugineo-fusca* (NF1411) | 12 | 0 | 27 | 5 | |
| *P. ferrugineo-fusca* (N16614) | 5 | 0 | 21 | 8 | |
| **P. ferrugineo-fusca* (N16621) | 17 | 0 | 31 | 0 | |
| **P. hypolateritia* (N9249) | 11 | 0 | 7 | 0 | |
| *P. incrassata* (NF7316) | 2 | 0 | 35 | 27 | A |
| **P. lenis* (B772) | 23 | 21 | 31 | 29 | A |
| **P. microspora* (N10724) | 0 | 0 | 25 | 22 | A |
| *P. pearsonii* (N9224) | 6 | 0 | 60 | 60 | A |
| **P. sequoiae* (N8755) | 2 | 0 | 13 | 10 | |
| **P. sitchensis* (B788) | 2 | 0 | 39 | 16 | |
| *P. spissa* (B791) | 12 | 13 | 38 | 34 | A |
| **P. subacida* (N9762) | 32 | 0 | 22 | 8 | |
| **P. subacida* (N16636) | 26 | 0 | 7 | 0 | |
| **P. unita* (N11259) | 8 | 0 | 54 | 55 | A |

TABLE 1 (*Continued*)

| Organism | Corn steep agar | | Thiamine peptone agar | | Acidity |
|---|---|---|---|---|---|
| | *Staph. aureus* | *E. coli* | *Staph. aureus* | *E. coli* | |
| **P. vaillantii* (N10444) | 5 | 0 | 14 | 8 | |
| **P. versipora* (B800) | 28 | 0 | 14 | 0 | |
| **P. vulgaris* (B802) | 0 | 0 | 12 | 0 | |
| **P. weirii* (N16606) | 16 | 26 | 17 | 35 | |
| **P. xantha* (N16570) | 18 | 14 | 40 | 38 | A |
| **P. xantha* (N16571) | 13 | 11 | 35 | 34 | A |
| **P. xantha* (N16629) | 0 | 0 | 30 | 23 | A |
| *P. xantha* (N16630) | 0 | 0 | 29 | 25 | A |
| *P. xantha* (N16645) | 5 | 0 | 35 | 35 | A |
| **P. xantha* (N16647) | 12 | 15 | 27 | 25 | A |
| **P. xantha crassa* (B805) | 17 | 20 | 35 | 35 | A |
| **P. xantha crassa* (B806) | 20 | 21 | 25 | 24 | A |
| **P. xantha crassa* (B807) | 12 | 6 | 46 | 40 | A |
| **P. xantha f. crassa* (B809) | 11 | 10 | 40 | 38 | A |
| **P. xantha f. crassa* (B810) | 0 | 0 | 21 | 16 | A |
| **Rhizoctonia crocorum* (NF6812) | 14 | 0 | 0 | 0 | |
| **Rhodopaxillus nudus* (L352) | 53 | 24 | 49 | 0 | |
| **Schizophyllum commune* (N16654) | 0 | 0 | 15 | 10 | |
| **Sparassis crispa* (N9244) | 0 | 0 | 18 | 10 | |
| **Stereum abietinum* (N11792) | 13 | 0 | 20 | 10 | |
| **S. frustulosum* (NF2055) | 0 | 0 | 24 | 10 | |
| **S. hirsutum* (N9282) | 16 | 10 | 18 | 11 | |
| **S. murrayi* (N11362) | 14 | 13 | 17 | 13 | |
| **S. purpureum* (NF682) | 0 | 0 | 16 | 0 | |
| **S. sanguinolentum* (N16618) | 21 | 20 | 12 | 7 | |
| **S. sulcatum* (N8116) | 42 | 30 | 28 | 22 | |
| **S. sulcatum* (N16563) | 28 | 17 | 49 | 43 | |
| **Stropharia mammillata* (L230) | 20 | 0 | 13 | 0 | |
| **S. merdaria* (L223.15) | 0 | 0 | 14 | 12 | |
| **Trametes americana* (N9414) | 24 | 19 | 18 | 0 | |
| **T. carbonaria* (N8432) | 11 | 8 | 11 | 10 | |
| *T. feei* (NF7320) | 7 | 7 | 41 | 42 | A |
| **T. heteromorpha* (N8440) | 24 | 0 | 42 | 37 | A |
| **T. heteromorpha* (N16558) | 46 | 4 | 50 | 50 | A |
| **T. heteromorpha* (N16620) | 30 | 9 | 32 | 35 | A |
| **T. isabellina* (B820) | 0 | 0 | 24 | 18 | |
| **T. isabellina* (B821) | 0 | 0 | 20 | 20 | |
| *T. lilacino-gilva* (NF7322) | 0 | 0 | 22 | 20 | A |
| *T. morganii* (B822) | 0 | 0 | 15 | 14 | |
| **T. serialis* (N10915) | 0 | 0 | 27 | 30 | A |
| **T. serialis* (N16561) | 15 | 9 | 50 | 50 | A |
| **T. serialis* (N16623) | 0 | 0 | 18 | 10 | |
| **T. serpens* (NF7343) | 65 | 25 | 45 | 37 | |
| **T. subrosea* (B833) | 2 | 4 | 27 | 20 | |
| **T. variiformis* (NF8046) | 0 | 0 | 34 | 25 | |
| **T. variiformis* (N16573) | 5 | 0 | 40 | 40 | A |
| **T. variiformis* (N16608) | 33 | 5 | 18 | 15 | A |
| **T. variiformis* (B835) | 26 | 14 | 30 | 25 | A |
| *Ungulina betulina* (L333) | 7 | 7 | 55 | 55 | A |

Several types of inhibition were observed. In one type the bacterial streak ended in a sharp distinct line with no evidence of growth between this

line and the edge of the fungus colony (fig. 1A, 1B). In a second, isolated colonies of bacteria appeared between the break in the streak and the fungus colony. These colonies were presumably resistant to the antibacterial material produced by the fungus (fig. 1D, 1E). In a third, the growth of the bacteria gradually tapered out toward the fungus colony (fig. 1C). A fourth type was observed in which the growth of the bacteria was uniformly faint for some distance between the fungus colony and the region of heavy growth of the bacteria. Variations of these main types appeared. For example, there occurred a combination of tapering growth with resistant colonies. Most of these types of inhibition were reported previously by Robbins *et al.* (7).

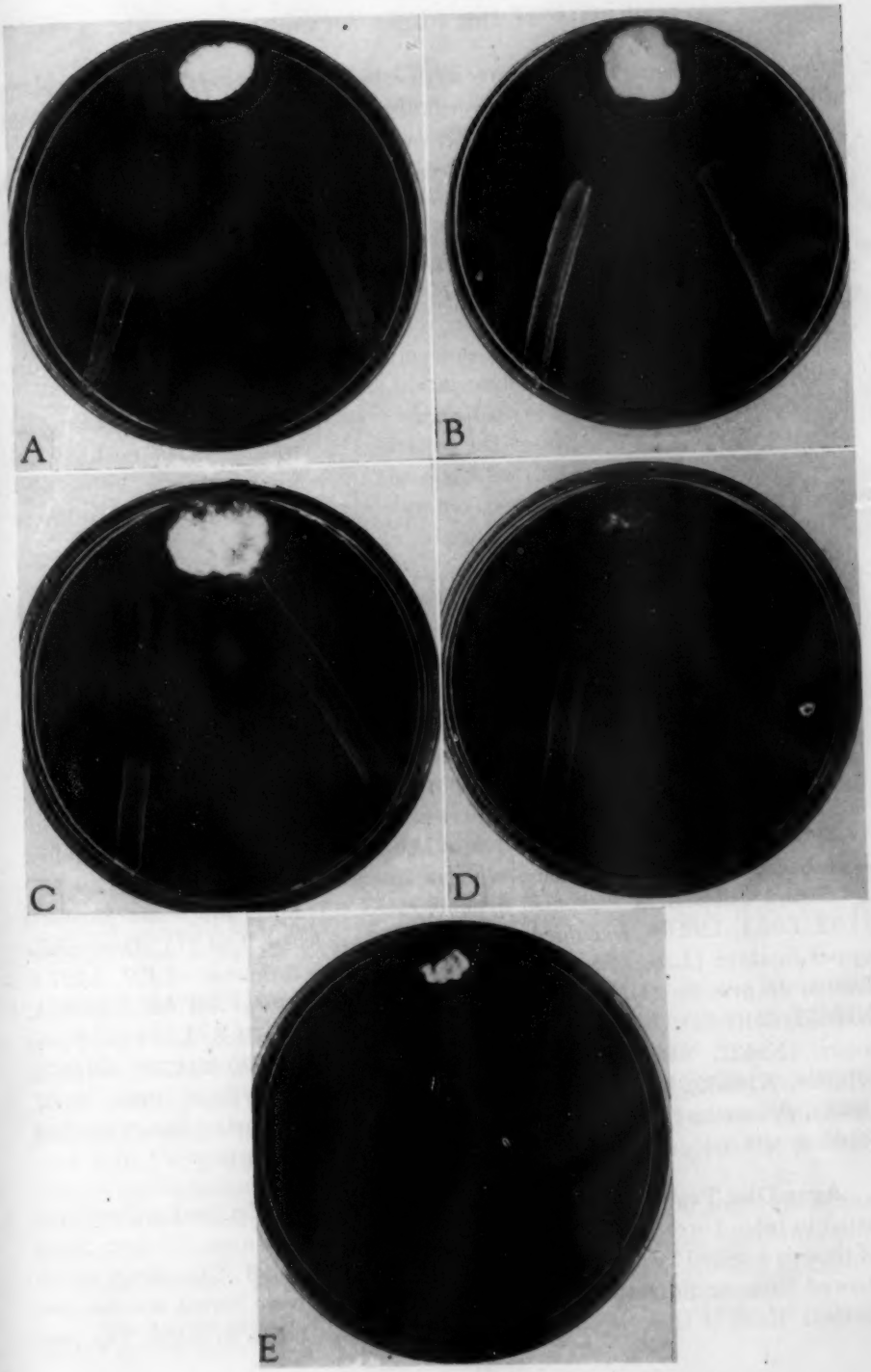
In table 1 the maximum zones of inhibition are recorded regardless of the character of the inhibition zone. The fungi which produced inhibition zones characterized by the presence of scattered individual resistant colonies were *Drosophila caudata* (L278), *D. exalbicans* (L450), *D. fibrillosa* (CBS23, L15.2), *D. semivestita* (L281), *D. trepida* (L247), *D. vernalis* (L367), *Fomes spadiceus* (N8102), *Peniophora incarnata* (NF8090), *Pleurotus griseus* (NF2948), and *Polyporus fragilis* (N10227). Fungi which were found to produce inhibition zones of the third type were *Alnicola escharoides* (L205), *A. melinoides* (L206), *Collybia dryophila* (L109), *Crepidotus mollis* (L73), *Fomes applanatus* (N9295), *F. fraxinophilus* (NF1921), *Panaeolus campanulatus* (L371), *Schizophyllum commune* (N16654), and *Sparassis crispa* (N9244). Inhibition zones of the fourth type were found with *Clitocybe ditopa* (L357), *Coprinus picaceus* (L14), *C. plicatilis* (L461), *Deconica inquilina* (L54b), *Irpex cinnamomeus* (NF2169), *Peniophora pubera* (NF3456), *Polyporus abietinus* (N16634), *P. giganteus* (NF3769), *P. zonalis* (NF2049), *Poria albobrunnea* (N16574), *P. weirii* (N16606), and *Trametes lilacino-gilva* (NF7322).

The antibacterial activity of most of the fungi investigated increased with the age of the fungus colony. However, the activity of some decreased with age. For example, the colony of *Polyporus osseus* (N11701) inhibited *Staph. aureus* for a distance of 25 mm. when tested after 12 days; a 19-day-old culture inhibited *Staph. aureus* for 3 mm.

As was observed earlier (7) there seemed to be for the fungi included in this investigation a negative correlation between rapidity of growth and antibacterial activity. Most of the fungi which grew rapidly showed no inhibitory effects and most of those which were most active were more slowly

**Explanation of figure 1**

A few types of inhibition obtained by the streak method. The plates were streaked with *Staph. aureus* (left) and *E. coli* (right). The colonies in A and B were grown on corn-steep agar, those in C, D, and E on thiamine peptone agar. A. *Drosophila subatrata* (L284.6), B. *Fomes annosus* (N8273), C. *Coprinus picaceus* (L14), D. *Drosophila fibrillosa* (L15), E. *Drosophila exalbicans* (L450). At the time of streaking the colony in A was eight days old. The other colonies were two weeks old.


A
B
C
D
E

growing organisms. Furthermore, as discussed later, observations were made which suggested that a particular organism might produce more antibacterial material in media on which its growth was less rapid.

None of the fungi tested was active against *E. coli* alone. The antibacterial activity of 55 fungi was confined to *Staph. aureus* with none observed on *E. coli*. When a fungus was effective against both bacteria there was greater activity against *Staph. aureus* than against *E. coli*. In a few exceptions, when the inhibition zone was larger with *E. coli* than with *Staph. aureus*, the difference was slight or the inhibition zone for *E. coli* was partial. It would require further investigation to determine whether these exceptions were valid or the result of individual variation.

The medium upon which a fungus grew influenced the antibacterial activity to some extent. Six of the isolations tested evidenced antibacterial activity when grown on the corn-steep agar but none on the thiamine peptone agar. Thirty-five fungi displayed antibacterial activity when they were grown on the thiamine peptone medium but they were not effective when cultivated on the corn-steep agar.

Different isolations of the same species of some of the fungi were found to vary markedly in their antibacterial activity. For example, a culture of *Trametes serpens* (NF7343) displayed good activity against both indicator bacteria by the streak method. Another isolation of the same species (B) showed no activity. Two isolations of *Schizophyllum commune* (L445 and N11795) gave negative results with both bacteria. A third isolation of the same species (N16654) inhibited both *Staph. aureus* and *E. coli* on the thiamine peptone agar. A culture of *Poria albobrunnea* (B738) showed no antibacterial activity. Another isolation of the same species (N16574) inhibited both bacteria on both the corn-steep and thiamine peptone media.

Strain differences were also demonstrated for *Agrocybe semiorbicularis* (L92, L92.1, L92.6), *Deconica inquilina* (L54a, L54b, L54.27), *Drosophila appendiculata* (L20, L25, L25.8, L84, L108), *D. exalbicans* (L127, L127.4, L450), *D. gracilis* (L171.4, L460), *Fomes nigrolimitatus* (B708, N10085A, N16651, N16652), *Lentinus tigrinus* (L38.1, L38.2, L38.3, L38.11), *Poria weirii* (N9422, N16606, N16607), *Poria xantha* (N9315, N16570, N16571, N16629, N16630, N16645, N16647), *P. xantha crassa* (B805, B806, B807, B808), *P. xantha* f. *crassa* (B809, B810, B811), *Stereum abietinum* (N11792, N16603, N16604), and *S. sulcatum* (N8116, N16563, N16619).

**Agar Disc Test.** One hundred and eighty-three of the most active fungi listed in table 1 were selected for further study with the agar disc test. Each of these is marked by an asterisk in table 1. In addition, 23 of the fungi which showed little or no activity by the streak method were tested by the disc method. Each of these is marked by an asterisk on pages 479–485. The pro-

cedure used for the agar disc test was the same reported previously (7) except that each plate of yeast peptone agar[7] was seeded with 0.04 ml. of a 6–8 hour AC broth culture of the indicator bacterium (fig. 2). *Staph. aureus* was used as the indicator organism for all the fungi tested by the disc method; disc tests were run with *E. coli* also for those fungi which had showed marked inhibition of this bacterium by the streak test.

**Results with the Agar Disc Test.** The results for the fungi tested with *E. coli* as the indicator organism were almost entirely negative. Zones of inhibition were produced by the discs from only three species: *Agrocybe dura* (L386.10, L386.14), *Drosophila semivestita* (N281), and *D. subatrata*

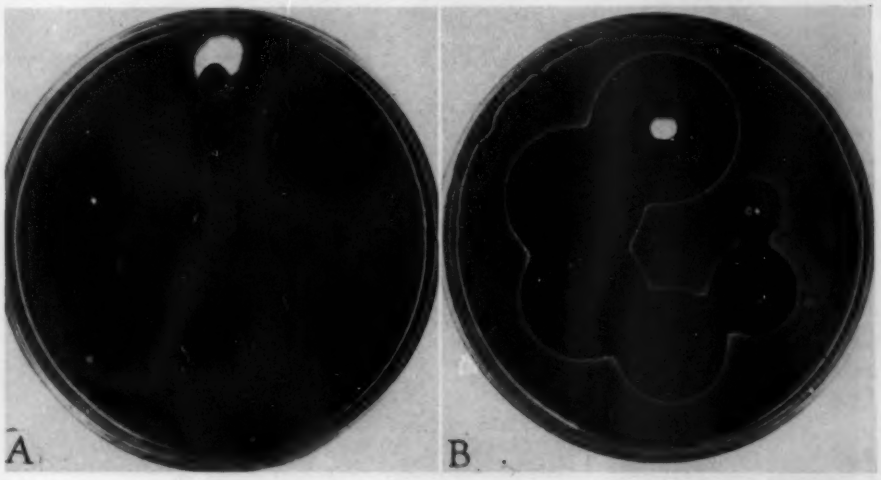



FIG. 2. The agar disc test with *Drosophila subatrata* (L284.6) grown on corn-steep agar for eight days. The discs cut from the culture in A were transferred to a yeast peptone medium seeded with *Staph. aureus*. The largest zone of inhibition in B was from the disc taken from nearest the center of the colony.

(L43.1, L284, L284.3, L284.6). The maximum inhibition zone was 9 mm. with *A. dura* and 15 mm. with *D. subatrata*. These zones showed partial inhibition, that is, contained numerous small colonies of bacteria.

Ninety fungi which had evidenced good activity by the streak test against *Staph. aureus* showed no activity against this bacterium by the agar disc test. Forty-eight isolations had some activity, producing zones with a maximum diameter of 10 mm.

The discs from 45 Basidiomycetes produced inhibition zones more than 10 mm. in diameter. The age and the medium on which the maximum zones of inhibition were produced by each of these fungi are recorded in table 2.

[7] The medium contained per liter 5 g. neopeptone, 3 g. yeast extract, 1 g. dextrose, and 15 g. Difco agar. The pH was 6.5 after autoclaving.

TABLE 2. *Antibacterial activity of fungi tested by the agar disc method.* The maximum inhibition zones in mm. against *Staph. aureus* are given. The age in days and medium on which the fungi were grown at the time of the test are indicated. C = corn-steep agar; P = thiamine peptone agar.

| Organism | Medium | Age | Zone |
|---|---|---|---|
| *Agrocybe dura* (L386.10) | C | 17 | 13 |
| *A. dura* (L386.14) | P | 23 | 11 |
| *Clitocybe obsoleta* (L358) | P | 13 | 22 |
| *C. robusta* (NF2932) | C | 16 | 11 |
| *Coprinus picaceus* (L14) | P | 12 | 20 |
| *Daedalea unicolor* (N10202) | P | 6 | 18 |
| *Drosophila caudata* (L278) | C | 12 | 13 |
| *D. exalbicans* (L450) | C | 23 | 15 |
| *D. fibrillosa* (CBS23) | C | 16 | 15 |
| *D. fibrillosa* (L15) | P | 17 | 17 |
| *D. fibrillosa* (L15.2) | C | 19 | 11 |
| *D. gracilis* (L171.4) | C | 28 | 14 |
| *D. semivestita* (L281) | P | 8 | 27 |
| *D. subatrata* (CBS25) | C | 16 | 28 |
| *D. subatrata* (L43.1) | C | 12 | 25 |
| *D. subatrata* (L122) | C | 13 | 25 |
| *D. subatrata* (L284) | P | 8 | 29 |
| *D. subatrata* (L284.3) | C | 13 | 27 |
| *D. subatrata* (L284.6) | C | 8 | 25 |
| *D. subatrata* (L449) | P | 10 | 28 |
| *D. vernalis* (L367) | P | 28 | 24 |
| *Fomes annosus* (N8273) | C | 20 | 16 |
| *Irpex mollis* (NF3047) | C | 16 | 13 |
| *Panaeolus papilionaceus* (L387.3) | C | 15 | 18 |
| *P. papilionaceus* (L387.9) | C | 13 | 15 |
| *Peniqphora incarnata* (NF8090) | C | 17 | 19 |
| *Pleurotus griseus* (NF2948) | C | 16 | 15 |
| *Polyporus amorphus* (N16597) | C | 38 | 13 |
| *P. dichrous* (N11609) | C | 8 | 17 |
| *P. montanus* (N8449) | C | 20 | 14 |
| *P. obtusus* (NF6798) | C | 20 | 23 |
| *P. radiatus* (N10229) | C | 20 | 11 |
| *P. tephroleucus* (?) (N10902) | C | 20 | 14 |
| *Poria hypolateritia* (N9249) | C | 12 | 13 |
| *P. sequoiae* (N8755) | C | 17 | 12 |
| *P. sitchensis* (B788) | P | 9 | 12 |
| *P. subacida* (N9762) | P | 6 | 15 |
| *P. subacida* (N16636) | C | 10 | 20 |
| *P. versipora* (B800) | C | 9 | 15 |
| *Rhodopaxillus nudus* (L352) | C | 13 | 20 |
| *Stereum frustulosum* (NF2055) | P | 6 | 12 |
| *S. sulcatum* (N8116) | C | 17 | 12 |
| *Trametes americana* (N9414) | C | 10 | 15 |
| *T. heteromorpha* (NF7343) | C | 17 | 17 |
| *T. heteromorpha* (N16558) | C | 13 | 15 |

Various types of inhibition were observed (fig. 3). Some of these were described in the earlier report (7). Burkholder (3) has reported on some of these growth patterns of bacteria obtained from testing various antibiotic substances, vitamins, and amino acids in glass cylinders placed on plates seeded with bacteria.

The degree and character of antibacterial activity varied with the

fungus, the medium, the age of the cultures, and the position of the disc in relation to the fungus colony.

The zones of inhibition listed in table 2 were partial for *Clitocybe obsoleta* (L358), *Coprinus picaceus* (L14), *Drosophila gracilis* (L171.4), *Polyporus amorphus* (N16597), *Poria subacida* (N9762, N16636), *P. versipora* (B800), and *Stereum sulcatum* (N8116). The fungi producing inhibition zones containing individual resistant bacterial colonies were *Drosophila exalbicans* (L450), *D. fibrillosa* (CBS23, L15), *D. vernalis* (L367), *Fomes annosus*

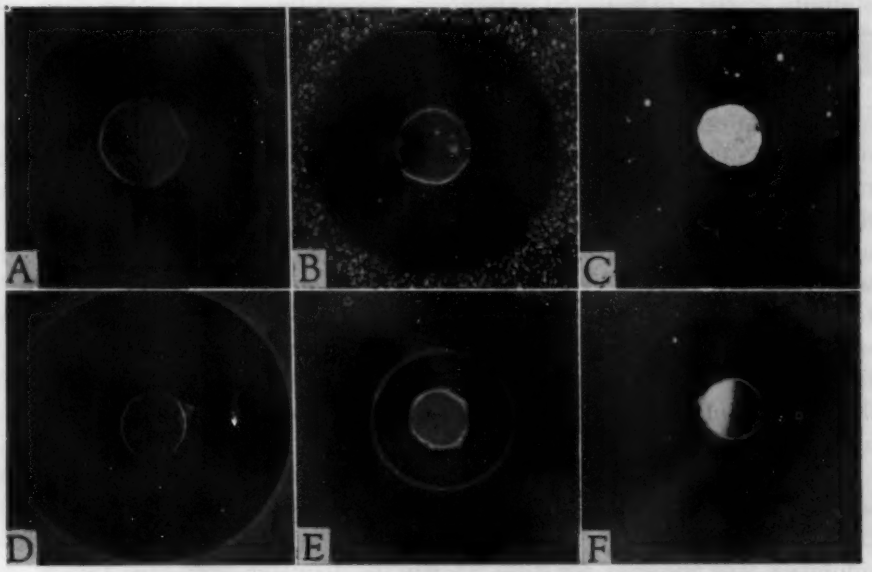



FIG. 3. Types of inhibition zones obtained by the agar disc method. The fungi were grown on corn-steep agar with the exception of *Drosophila subatrata* (L284.6) which was cultivated on the thiamine peptone agar. The disc in C was taken from the fungus colony; the discs in A, B, D and E were cut from outside the limits of the colony; half of the disc in F is from the colony. A. *Drosophila subatrata* (L284.6), B. *Fomes annosus* (N8273), C. *Drosophila fibrillosa* (L15), D. *Drosophila subatrata* (L449), E. and F. *Poria subacida* (N9762). The zone in A shows complete inhibition of *Staph. aureus;* B and C show types of inhibition zones characterized by scattered individual resistant colonies and partial inhibition; in D a zone of stimulation surrounds the inhibition zone; E shows alternating zones of more or less marked inhibition; in F a zone of partial inhibition surrounds the zone of marked inhibition; the inhibition zone in F is asymmetrical with greater inhibition on the side of the disc containing mycelium.

(N8273), *Panaeolus papilionaceus* (L387.3, L387.9), *Polyporus tephroleucus* (?) (N10902), *Rhodopaxillus nudus* (L352), and *Trametes americana* (N9414). The zones produced by discs from the other fungi tested were clear.

With most of the fungi tested the greatest activity was produced by the disc taken from nearest the center of the fungus colony. With a few of the fungi tested, for example, *Poria versipora* (B800) and *P. subacida* (N16636),

the disc with greatest activity was the one with the youngest mycelium, that taken from the periphery of the colony. The discs from just outside the limits of the colony of *Agrocybe dura* (L386.14) grown on the thiamine peptone agar for 23 days produced larger inhibition zones than those from within the mycelial colony. The antibacterial activity of *Clitocybe robusta* (NF2932) and *Pholiota lucifera* (NF673) was confined to the discs taken from the colonies. The differences in the activity of discs cut from various parts of the fungus colony or outside of it are doubtless correlated with the stability of the antibacterial substances, the readiness with which they are excreted by the fungus, the rate at which they diffuse in the agar, and other factors which were not analyzed in this study.

**Correlation between the Streak and Disc Tests.** As noted in the previous report (7) the correlation between the results obtained from the streak method and the disc method was not always satisfactory. A fungus which displayed good antibacterial activity by the streak test might evidence little or no activity by the disc test. Various explanations for this discrepancy were considered and some of them were tested experimentally. In the streak test we are dealing with materials produced by an entire fungus colony which has been growing for several days or even weeks. Whereas in the disc test the antibacterial material must be sufficiently concentrated in and diffusible from an agar disc 5.5 mm. in diameter to produce its effect within a few hours.

*Trametes serpens* (NF7343) evidenced marked activity by the streak method when grown on the corn steep medium. Discs of various sizes cut from the fungus colony were tested against *Staph. aureus*. Practically no inhibition was observed for discs 5.5 mm. in diameter, but discs 15 mm. in diameter showed distinct inhibition of the bacteria, and the size of the inhibition zones was directly correlated with the size of the discs.

Another factor considered was the slowness of diffusion of the antibacterial substances. The activity of discs placed on a yeast peptone agar seeded with *Staph. aureus* and kept at 15° C for 4.5 hours before incubation at 37° C was compared with that of similar discs incubated immediately at 37° C.

For six fungi tested in this way there was no difference in the activity of the discs incubated immediately and those from which the material was permitted to diffuse before incubation. For others the inhibition shown was greater for the discs first kept at 15° for 4.5 hours. For example, an agar disc from *Merulius lachrymans* kept at 15° C for 4.5 hours before incubation at 37° produced an inhibition zone measuring 19 mm., as compared with a zone of 11 mm. produced by a similar disc on a plate incubated immediately at 37° C.

Another factor involved was the test medium. In the streak test the indicator organisms were grown on the thiamine peptone or corn-steep medium on which the fungi were cultivated. The disc tests were carried out on a yeast peptone medium seeded with the indicator bacteria.

The effect of the test medium was determined for twenty fungi which gave inhibition zones of at least 10 mm. when streaked with *Staph. aureus*. Agar discs from 23-day-old cultures grown on the corn-steep and thiamine peptone media were placed on three media equally seeded with *Staph. aureus*.

TABLE 3. *Effect of the test medium on antibacterial activity by the agar disc method.* The organisms were grown for 23 days on the corn-steep and thiamine peptone agar. Similar discs were tested on three media equally seeded with *Staph. aureus*. C = corn-steep agar; P = thiamine peptone agar; Y = yeast peptone agar. Maximum inhibition zones in mm.

| Organism | Grown on C | | | Grown on P | | |
|---|---|---|---|---|---|---|
| | Discs tested on | | | | | |
| | C | P | Y | C | P | Y |
| *Clitocybe robusta* (NF2932) | 30 | 25 | 15 | 22 | 18 | 9 |
| *Coniophora puteana* (N9779) | 11 | 15 | 0 | 0 | 7 | 0 |
| *Coprinus comatus* (NF2935) | 9 | 8 | 0 | 15 | 12 | 0 |
| *Irpex mollis* (NF3047) | 12 | 11 | 13 | 16 | 14 | 11 |
| *Lenzites bicolor* (NF7326) | 0 | 0 | 7 | 0 | 0 | 0 |
| *Merulius lachrymans* (NF3526) | 10 | 7 | 0 | 24 | 22 | 11 |
| *Paxillus panuoides* (NF3007) | 8 | 11 | 0 | 10 | 11 | 0 |
| *Pholiota lucifera* (NF673) | 18 | 18 | 11 | 15 | 19 | 8 |
| *Pleurotus griseus* (NF2948) | 16 | 13 | 12 | 11 | 8 | 7 |
| *Polyporus amorphus* (N8552) | 16 | 15 | 0 | 9 | 10 | 0 |
| *P. caesius* (NF6891) | 9 | 0 | 0 | 0 | 0 | 0 |
| *P. semipileatus* (NF3499) | 20 | 10 | 8 | 15 | 14 | 8 |
| *Trametes carbonaria* (N8432) | 12 | 9 | 0 | 8 | 8 | 0 |
| *T. serpens* (NF7343) | 15 | 8 | 6 | 0 | 0 | 0 |
| *T. variiformis* (NF8046) | 7 | 0 | 0 | 0 | 0 | 0 |

The three media included the corn-steep, thiamine peptone, and yeast peptone agars. The maximum inhibition zones in millimeters obtained from a series of three discs cut on a radius starting at the center of the colony are recorded in table 3.

The activity of the discs was greatest for most of the fungi tested on the corn-steep test medium and least on the yeast peptone medium. This may be associated with the higher pH of the yeast peptone medium[8] or the fact that it is better for the growth of *Staph. aureus* than the corn-steep medium. However, this was not true with all the fungi. A disc from a culture of *Lenzites bicolor* (NF7326) which gave no activity on the corn-steep or thiamine peptone media showed slight activity on the yeast peptone agar. The activity of the discs from cultures of *Coniophora puteana* (N9779) and

[8] The pH of the yeast peptone agar was 6.5 as compared with a pH of 5.7 for the corn-steep agar and a pH of 5.5 for the thiamine peptone agar.

*Pholiota lucifera* (NF673) was best on the thiamine peptone test medium. The discs from cultures of *Corticium portentosum* (N9283), *Omphalia flavida* (NF5711), *Polyporus mollis* (NF6827), *P. nidulans* (N9312), and *Poria candidissima* (N11613) were inactive on the three test media.

It was observed that the test medium sometimes affected the character of the inhibition zone. For example, discs from a culture of *Merulius lachrymans* (NF3526) grown on the thiamine peptone agar produced clear zones on the yeast peptone agar, but produced larger zones of partial inhibition on both the corn-steep and thiamine peptone media.

The three test media used above varied in their sugar content. The corn-steep agar contained 40 g. of dextrose per liter; the thiamine peptone agar 10 g. per liter; the yeast peptone one gram per liter. By increasing the dextrose in this medium to 40 g. per liter the activity of the discs of *Clitocybe robusta* (NF2932) and *Polyporus semipileatus* (NF3499) on the yeast peptone agar was increased. The inhibition zones on the yeast peptone agar plus added dextrose, however, were not so large as the zones produced by comparable discs on the corn-steep test medium. Substances other than sugar alone are evidently concerned with the differences obtained with the various test media.

It seems probable that no single explanation can account for all those instances in which marked activity was observed by the streak test and little or none by the disc test and the relative importance of the different factors probably varies with the fungus. In general the disc test on the yeast peptone medium puts the antibacterial substances to a more severe test.

A few fungi which displayed negative or almost negative antibacterial activity by the streak test gave some activity with the disc test. For example, discs cut from the colony of *Lenzites saepiaria* (N9528) grown on the corn-steep agar for 20 days produced inhibition zones as large as 13 mm. in diameter with *Staph. aureus*. Similar observations were made with *Polyporus tulipiferus* (NF8049). It appears that the active material was largely retained by the mycelium and released upon the cutting of the discs.

**Liquid Cultures and the Serial Dilution Method.** Fifty-three of the fungi found to have the most antibacterial activity by the streak and disc tests were grown in liquid culture. Each fungus was usually grown at 25° C in Fernbach flasks, each containing one liter of medium and 4–6 coils of beech shavings. The corn-steep and thiamine peptone media used for the streak and disc tests were prepared without agar. When the mycelial mat had grown on the surface of the liquid a sample of the culture fluid was assayed against *Staph. aureus* by the serial dilution method used in this laboratory (5).[9]

[9] The assistance of Mrs. Catherine Amos in these tests is acknowledged.

Samples of the culture fluids were taken at intervals depending upon the rapidity of growth of the fungus. This was usually after a 1–4-week period. If the fluid was active, another sample was usually taken in a few days or a week later. If it was apparent that there was no further increase in titer with age, the culture fluid was poured out of the flask and a liter of fresh sterile medium was added so that the mycelial mat could come in contact with it. For some of the most active fungi only a few days contact was necessary in order to obtain a culture fluid with suitable antibacterial activity. A mat could be reflooded several times by this method.

The following fungi grown on the corn-steep or thiamine peptone media produced fluids with a maximum activity of 32 dilution units or less against *Staph. aureus:*

*Agrocybe dura* (L386.14)
*Clitocybe robusta* (NF2932)
*Coniophora puteana* (N9779)
*Coprinus comatus* (NF2935)
*C. picaceus* (L14)
*Corticium portentosum* (N9283)
*Drosophila fibrillosa* (L15, L15.2)
*D. vernalis* (L367)
*Irpex mollis* (NF3047)
*Lenzites bicolor* (N7326)
*Merulius lachrymans* (NF3526)
*Omphalia flavida* (NF5711)
*Panaeolus papilionaceus* (L387.3, L387.9)
*Paxillus panuoides* (NF3007)
*Peniophora incarnata* (NF8090)
*Pholiota lucifera* (NF673)
*Polyporus amorphus* (N8552)
*P. caesius* (NF6891)
*P. dichrous* (N11609)
*P. mollis* (NF6827)
*P. montanus* (N8449)
*P. nidulans* (N9312)
*P. obtusus* (NF6798)
*P. radiatus* (N10229)
*P. semipileatus* (NF3499)
*Poria candidissima* (N11613)
*P. sequoiae* (N8755)
*P. subacida* (N9762)
*Stereum frustulosum* (NF2055)
*S. sulcatum* (N8166)
*Trametes americana* (N9414)
*T. carbonaria* (N8432)
*T. heteromorpha* (N16558)
*T. serpens* (NF7343)
*T. variiformis* (NF8046)

Culture fluids with activity of 64 dilution units or more were obtained from sixteen isolations as recorded in table 4. The second column gives the maximum activity in dilution units against *Staph. aureus.* The third column shows whether the greatest activity was obtained with the corn-steep (C) or thiamine peptone (P) medium. The fourth column records the type of container in which the cultures were growing at the time of the maximum activity of the culture fluid. These were either Fernbach flasks (F) containing one liter of the solution and beech shavings or 250-ml. wide-mouthed Erlenmeyer flasks (250) each with 100 ml. of the medium and beech shavings. The last column gives the age of the culture fluid at the time its greatest activity was observed. The most active culture fluids obtained with three of the fungi were the result of reflooding the mycelial mats. The culture of *Trametes heteromorpha* (N8440) was reflooded with fresh sterile solution after one month's growth. The mats of *Clitocybe obsoleta* (L358) and *Rhodopaxillus nudus* (L352) had grown a little more than three months before reflooding.

TABLE 4. *Antibacterial activity of fungi grown in liquid cultures.* Maximum activity in dilution units. C = corn-steep liquid; P = thiamine peptone liquid; F = Fernbach flask; 250 = 250 ml. wide-mouthed Erlenmeyer flask. See text for details.

| Organism | Maximum activity dilution units | Medium | Container | Age |
|---|---|---|---|---|
| *Agrocybe dura* (L386.10) | 512 | C | F | 6 weeks |
| *Clitocybe obsoleta* (L358) | 128 | C | 250 | 6 week reflood |
| *Daedalea unicolor* (N10202) | 256 | P | 250 | 4 weeks |
| *Drosophila semivestita* (L281) | 64 | C | F | 4 weeks |
| *D. subatrata* (CBS25) | 2048 | C | F | 2 months |
| *D. subatrata* (L43.1) | 1024 | C | F | 4 weeks |
| *D. subatrata* (L122) | 256 | C | F | 7 weeks |
| *D. subatrata* (L284) | 64 | C | F | 5 weeks |
| *D. subatrata* (L284.3) | 1024 | C | F | 4 weeks |
| *D. subatrata* (L284.6) | 2048 | C | F | 5 weeks |
| *D. subatrata* (L449) | 64 | C | F | 2 months |
| *Fomes annosus* (N8273) | 128 | C | 250 | 12 days |
| *Poria hypolateritia* (N9249) | 128 | C | 250 | 16 days |
| *P. sitchensis* (B788) | 64 | C | 250 | 16 days |
| *Rhodopaxillus nudus* (L352) | 1024 | C | F | 16 day reflood |
| *Trametes heteromorpha* (N8440) | 64 | C | F | 17 day reflood |

Very active culture fluids were obtained from several isolations of *Drosophila subatrata*. Culture fluids active at a dilution of 1 to 2048 were obtained with CBS25 and L284.6. A 20-day-old mycelial mat (L284.6) reflooded with the corn-steep medium produced a liquid active at a dilution of 1 to 1024 after six days.

A 48-day-old mycelial mat of *Agrocybe dura* (L386.10) produced a culture liquid active at a dilution of 1 to 256 ten days after reflooding with the corn-steep liquid. Reflooding a mycelial mat of *Rhodopaxillus nudus* (L352) which had previously grown for three months in a 250-ml. flask with the corn-steep liquid produced a fluid active at a dilution of 1 to 512 after five days.

**Trametes serpens (NF7343).** In the course of this study it was noted that several fungi showing excellent antibacterial activity by the streak test produced disappointing results by the disc test. One of the fungi which appeared to be particularly worthy of further investigation was *Trametes serpens* (NF7343). When this organism was grown on the corn-steep agar, inhibition zones by the streak test were observed measuring 65 mm. on *Staph. aureus*, 25 mm. on *E. coli*, and 20 mm. on *Pseudomonas aeruginosa*. On the thiamine peptone agar inhibition zones of 45 mm. on *Staph. aureus*, 37 mm. on *E. coli*, and 20 mm. on *P. aeruginosa* were obtained. The growth on the corn-steep medium was very slow, the radius of the colony measuring about 3 mm. after two weeks. Growth on the thiamine peptone agar was more rapid, the radius measuring 22 mm. after two weeks. A very slight amount of

acid was produced by the fungus. On a tryptose[10] agar the fungus inhibited *Mycobacterium phlei* for 25–28 mm. However, colonies showing good inhibition by the streak test gave negative results with the disc test.

How far the composition of the medium on which the fungus was grown affected the antibacterial activity against *Staph. aureus* was determined by the agar disc test. A variety of natural media and synthetic media supplemented in different ways was used. The pH of some of the media was adjusted to determine the effect of hydrogen ion concentration. A preliminary experiment indicated that the organism grew best at 25° C and this temperature was used throughout the experiments described below.

The corn-steep agar was varied by substituting $K_2HPO_4$ for the $KH_2PO_4$ in order to make the medium more alkaline. This medium was also used with the addition of 2 ml. potato extract[11] per l. Agar discs were cut from colonies 5 weeks old. The potato extract reduced the growth of the organism on the alkaline corn-steep agar; the colony was compact, brownish, with a radius of 7 mm. The maximum inhibition zone from discs taken from this culture was 14 mm. in diameter and a disc cut more than 20 mm. from the edge of the colony produced an inhibition zone of 11 mm. On the alkaline corn-steep agar the mycelium was white and the colony radius was 25 mm. The maximum inhibition zone by the disc test was 10 mm. and the activity was confined to the discs with mycelium.

*Trametes serpens* was grown on various media in Petri dishes to determine the effect of medium on growth and antibacterial activity. Supplementary observations were made on the growth of the fungus on the various media in test tubes. All media were prepared with 15 g. Difco agar per l. These included (a) 2 per cent malt extract, (b) 2 per cent malt extract treated with Norit A, (c) 5 per cent malt extract, (d) 2 per cent malt extract to which was added malt eluate[12] equivalent to 3 per cent malt extract, (e) a thiamine peptone stock culture medium containing per liter 1.5 g. $KH_2PO_4$, 0.5 g. $MgSO_4 \cdot 7H_2O$, 50 g. dextrose, 1 g. neopeptone, 2 g. asparagine, 0.5 ml. mineral supplements, and 500 mμmoles thiamine, (f) a tryptose medium containing per liter 10 g. Bacto tryptose, 10 g. dextrose, 2.5 ml. of a vitamin mixture,[13] and 1.25 γ biotin, (g) the same with malt eluate, (h) the AC medium (see 5), (1) the same plus malt eluate equivalent to 5 per cent malt extract, (j) a basal medium containing per liter 1.5 g. $KH_2PO_4$, 0.5 g. $MgSO_4 \cdot 7H_2O$, 50 g. dextrose, 2 g. asparagine, 0.5 ml. mineral supplements,

[10] The medium contained per liter 20 g. Bacto tryptose, 10 g. dextrose, 15 g. Difco agar, 2.5 ml. of a vitamin mixture (see footnote 13), and 1.25 γ biotin.

[11] The dry weight of the potato extract was 594.8 mg./ml.

[12] The malt eluate was prepared by extracting the Norit A with ammoniacal acetone.

[13] The mixture contained per ml. 100 mμmoles each of p-aminobenzoic acid, calcium pantothenate, guanine, hypoxanthine, 2-methyl-1,4 naphthohydroquinone diacetate, pimelic acid, nicotinamide, pyridoxine, riboflavin, thiamine, and 100,000 mμmoles i-inositol.

12 ml. of a vitamin mixture,[13] 6 γ biotin, and 625 mg. casein hydrolysate (the pH was raised to 5.8 with NaOH), (k) the same with added Hutchings synthetic casei factor (Lederle) 120 γ per liter, (l) k with 1200 γ folic acid (R. J. Williams π 4000) per liter.

The growth of the fungus was best on medium (a) and least on (e), (h), (i), (j), (k), and (l). Colonies grown for 16 days in Petri dishes on these different media were tested against *Staph. aureus* using the agar disc method. Negative results were obtained from colonies grown on (e), (h), and (i). The largest and clearest zones of inhibition, measuring 19 mm. in diameter as a maximum, were obtained from colonies grown on (j), (k), and (l). The zones from (b) were as large but the inhibition was partial.

Since medium (j) appeared to be the most satisfactory for the production of antibacterial activity, further experiments were carried on with this medium in which the sources of nitrogen and amounts of dextrose were varied. The nitrogen sources used at 1, 2, and 4 g. per liter included casein hydrolysate, asparagine, neopeptone, synthetic gelatine hydrolysate, a mixture of five amino acids,[14] and another mixture of six amino acids.[15] The amounts of dextrose used were 10, 20, and 50 g. per liter.

The growth of *Trametes serpens* was best with the various amounts of the synthetic gelatine hydrolysate and with 4 g. neopeptone per liter. After two weeks, agar discs from the colonies grown on the various media were tested against *Staph. aureus* by the agar disc test. Negative or nearly negative results were obtained from the colonies grown on both mixtures of amino acids and the synthetic gelatine hydrolysate. Zones with partial inhibition were observed for the discs from the colonies grown on the casein hydrolysate and neopeptone. The largest and clearest inhibition zones were obtained from the cultures grown on the asparagine media. The best medium for antibacterial activity appeared to be the basal medium prepared with 2 g. asparagine and 50 g. dextrose per liter. When this medium was used, an inhibition zone 21 mm. in diameter was produced. The antibacterial material diffused readily in sufficient concentration so that a disc cut more than 20 mm. from the edge of the fungus colony produced an inhibition zone 10 mm. in diameter. The growth of the fungus on this medium was slow, the colony radius measuring 3 mm. after two weeks.

These experiments on the effect of the culture medium showed that, in general, the greatest antibacterial activity was obtained on those media on which the fungus grew slowly. The asparagine medium appeared to be the most satisfactory. A culture fluid with an activity of 64–128 dilution units against *Staph. aureus* was obtained after one month's growth of the fungus

[14] These included d-arginine, dl-alanine, dl-aspartic acid, glycine, and d-glutamic acid.

[15] These included l-leucine, d-isoleucine, dl-phenylalanine, d-glutamic acid, l-tyrosine, d-arginine, and were prepared so that they were in the same proportions as in casein.

in the liquid asparagine medium in a 500-ml. Erlenmeyer flask or in a 250-ml. wide-mouthed flask.

Since *Trametes serpens* grew best on a 2 per cent malt agar but showed its greatest antibacterial activity on the synthetic medium described above prepared with 2 g. asparagine and 50 g. dextrose per liter, it was hoped that an active culture fluid would be obtained by growing the fungus in a 2 per cent malt liquid until the formation of a thick mycelial mat and then replacing the malt liquid with the asparagine medium. The results were disappointing. The highest activity obtained by reflooding a mycelial mat grown in a Fernbach flask with the asparagine medium was 16 dilution units. Culture fluids with a maximum activity of 16 dilution units were also obtained by reflooding mats previously grown on 2 per cent malt with the corn-steep medium, a corn-steep medium prepared with $K_2HPO_4$, and the same with added potato extract (2 ml. per l.).

## DISCUSSION

Antibacterial activity was found to be widespread in the group of Basidiomycetes included in this investigation. About 55 per cent of the 508 isolations investigated by the streak method showed antibacterial activity against *Staph. aureus* or against *Staph. aureus* and *E. coli*. This percentage of active isolations compares favorably with those of other investigations on the Basidiomycetes and other groups of fungi. Strict comparisons would not be valid, considering the difference in methods used by the various investigators.

Only 17 of the 64 genera included in this survey demonstrated no antibacterial activity by the streak method and 14 of these genera were represented by a single species. For 6 genera, each represented by 29 or more isolations, the number of isolations which were negative, moderately active or strongly positive is given in table 5, which includes in the last column the number observed to produce acid which might account for the antibacterial activity observed. Of the six genera, *Drosophila* and *Trametes* showed the largest

TABLE 5. *Distribution of antibacterial activity in 6 genera surveyed by the streak method.* A rating of strongly positive was given fungi giving inhibition zones of more than 10 mm. on *Staph. aureus*.

| Genus | Total number of isolations | Negative | Moderately active | Strongly positive | Acid producers |
|---|---|---|---|---|---|
| *Coprinus* | 38 | 31 | 2 | 5 | 0 |
| *Drosophila* | 48 | 16 | 9 | 23 | 0 |
| *Fomes* | 40 | 19 | 6 | 15 | 6 |
| *Polyporus* | 94 | 51 | 8 | 35 | 12 |
| *Poria* | 80 | 31 | 16 | 33 | 17 |
| *Trametes* | 29 | 5 | 5 | 19 | 10 |

proportion of active isolations. However, a considerable number in these genera were isolations of the same species.

It need not be pointed out that the results obtained on antibacterial activity depend on the methods of investigation as well as the fungi investigated. The streak test has definite limitations. It does not detect antibacterial substances produced and retained within the mycelium. The number of fungi among those investigated which produced intracellular antibacterial substances was not determined by the method of survey used. Observations on *Lenzites saepiaria* (N9528) and *Polyporus tulipiferus* (NF8049), which were negative by the streak test but positive by the disc test, in which the injury to the mycelium in cutting the discs might permit intracellular antibacterial substances to diffuse, emphasize the limitation of the streak method discussed here.

It is possible that antibacterial activity might have been found in a number of the fungi included in this investigation if extracts of the mycelium had been made and tested for activity against the indicator bacteria. However, from a practical standpoint, that is, the production of sufficient antibacterial material to permit concentration and possible isolation, the intracellular substances are of less importance than the extracellular. It would be more difficult to grow sufficient mycelium for extraction purposes than to deal with culture liquid.

The streak method is further complicated because of the effect of hydrogen ion concentration, resulting from organic acids produced by the metabolism of the fungus and by the possible exhaustion of nutrients essential for the growth of the bacteria in the vicinity of the fungus colony. The hydrogen ion concentration was taken into account by suitable tests at the time the plates were streaked. However, the possible importance of the exhaustion of essential nutrients in the vicinity of the colony could not be estimated in this investigation by the streak method. It should be pointed out, however, that neither of these factors is of importance when the disc test is used, because the yeast peptone agar upon which the discs were placed furnishes an adequate medium for the indicator bacteria and because its buffer action neutralizes the small amount of acid which might diffuse from the agar disc.

The medium on which the fungus is grown is another factor of importance in determining its antibacterial activity. This was illustrated by the studies on *Trametes serpens* and by the comparisons between corn-steep agar and thiamine peptone agar for a number of fungi investigated. It is possible that some of the Basidiomycetes included in this study would evidence activity if cultivated on some other medium. In this investigation the two media on which all organisms were tested were chosen because they permitted growth of the fungi and of the indicator bacteria, thus avoiding the

necessity of coating the agar on which the fungi had grown with a medium suitable for the growth of the indicator bacteria.

Evidence of antibacterial activity depends also upon the indicator organisms. *Staph. aureus* and *E. coli* represented the Gram-positive and Gram-negative bacteria as in the work of most other investigators. It is recognized, however, that because of the specificity in the action of antibacterial substances, the best indicator organism to employ is the one for which an antibiotic substance is desired. It would be desirable to re-survey the fungi included in this investigation using other indicator bacteria, especially those belonging to the acid-fast group.

The age of the fungus colony is also important in an investigation of antibacterial activity. Most of the organisms were tested at two ages. These results show that antibacterial activity may disappear with age, or it may increase with age, depending upon the fungus and the conditions under which it is grown. Here again, it is possible that more extensive search than time permitted might change some fungi from the negative to the positive class.

The results depend also on the strain of the fungus investigated. This has been demonstrated by others for various fungi including *Penicillium notatum* and other species found to produce antibiotic substances. Differences in the antibacterial activity among several isolations of the same species were also found in this survey. Certain isolations of *Trametes serpens, Schizophyllum commune,* and *Poria albobrunnea* were inactive. Other isolations of these same species evidenced antibacterial activity. Strain differences were also demonstrated for eleven other species included in this report.

When results of the present survey are compared with those of other investigators, differences are found. The present survey included 66 species reported on by Wilkins and Harris (12). Nineteen of these were negative according to both studies. Six species found active by Wilkins and Harris gave negative results in this survey. Twenty-nine reported by Wilkins and Harris to be negative displayed varying degrees of antibacterial activity in this survey. Sixteen of the species reported on by Mathieson (6) were included in this report. Five species which he found negative gave zones of inhibition of more than 10 mm. by the streak method. The results with the other 11 species were similar in both reports. Fifty-three species reported here were surveyed by Wilkins (9). Of 9 species with strongly positive results in his report, one was negative in this survey. Of 33 species found by Wilkins to be negative, 7 were found to be active. Of 76 species reported by Robbins and his associates (7) to give negative results, 13 were found to produce inhibition zones of more than 10 mm. in this investigation. Seven of the 58 species found by Robbins and associates to produce inhibition zones of more than 10 mm. gave negative results in this survey.

Differences in antibacterial activity found by various workers may be accounted for to some extent by differences in the fungi employed. There is no guarantee that the organisms studied under a particular species name were identical as to species or strain. In addition, the methods of investigation vary. For example, Wilkins and Harris and Mathieson used extracts of the sporophores for their investigations; Robbins and his colleagues used the streak method but the fungi were grown on the thiamine peptone medium and a 2 per cent malt agar. To evaluate the relative importance of fungi and methods in determining the differences in the results of various investigators it would be necessary to obtain subcultures of the particular isolations used in one laboratory and study them in another.

A number of the Basidiomycetes surveyed in this paper seem to be worthy of further investigation, in particular, *Drosophila subatrata*, *Rhodopaxillus nudus*, and *Agrocybe dura*. Whether or not any of them became therapeutically important can only be determined by further research.

## SUMMARY

About 55 per cent of the 508 isolations of Basidiomycetes investigated by the streak method showed antibacterial activity against *Staph. aureus* or against *Staph. aureus* and *E. coli*. None was active against *E. coli* alone. Two hundred and three inhibited the growth of *Staph. aureus* for more than 10 mm. by this method. For about 25 per cent of these, the inhibition was probably caused by the hydrogen ion concentration. Forty-five isolations produced inhibition zones more than 10 mm. in diameter by the agar disc method. Culture fluids with activity of 64 dilution units or more were obtained from sixteen isolations. Culture fluids active at a dilution of 1024–2048 were obtained with several isolations of *Drosophila subatrata* and *Rhodopaxillus nudus*. With several fungi there appeared to be a negative correlation between growth and antibacterial activity.

The author wishes to express her gratitude to Dr. William J. Robbins for his advice, active interest, and assistance throughout the course of this investigation.

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# OBSERVATIONS ON STREPTOMYCES GRISEUS—I. CHEMICAL CHANGES OCCURRING DURING SUBMERGED STREPTOMYCIN FERMENTATIONS

EUGENE L. DULANEY AND D. PERLMAN

In order to control a fermentation it is helpful, if not necessary, to understand its mechanism; these studies of the various factors influencing streptomycin production have therefore included an investigation of the chemical changes occurring during the fermentation.

A review of the literature indicates that knowledge of the metabolic activities of the actinomycetes in surface or submerged culture is quite limited. Schatz and his co-workers (1944) and Waksman and others (1946) have described in some detail the changes occurring during the production of streptomycin in surface culture but did not investigate the submerged fermentation so extensively. Somewhat earlier Woodruff and Foster (1943) reported a few of the changes occurring during the production of streptothricin and also actinomycin. The following experiments were conducted preparatory to investigating other factors governing the course of the fermentation on natural and synthetic media.

## METHODS

**Cultural:** An isolate from a culture originally obtained from Dr. S. A. Waksman's laboratory was used in these studies. Stock cultures were maintained by mixing spores with sterile soil. Inoculum was developed by spreading spores from the soil tubes over a Blake bottle slant of yeast extract—glucose agar medium. These cultures were incubated at 28° C until sporulation had occurred; cultures not used immediately were refrigerated. Fifty ml. of sterile distilled water were added to each bottle and a spore suspension was prepared by scraping with a sterile wire needle. One ml. of this spore suspension served as inoculum for each fermentation flask.

Unless otherwise indicated the basal medium contained 10 g. glucose, 5 g. peptone, 5 g. sodium chloride, 5 g. meat extract, and sufficient distilled water to bring the volume to 1 l. This is the medium used by Waksman and his co-workers (1946). Forty ml. aliquots were distributed in 125 ml. Erlenmeyer flasks, the flasks were plugged with cotton, and sterilized at 121° C for 20 min. They were inoculated with the spore suspension when cool and then placed on a rotary shaker. The shaker imparted a circular motion to the flasks such that all points on the flask described a circle of 1.5 in. diameter; the speed was 220 rpm. This shaker was located in a

constant temperature room maintained at 28° C. At the end of each fermentation interval two or three replicate flasks were removed and the contents pooled for analysis.

**Analytical:** The mycelium was removed from an aliquot of the fermented medium and the cell-free solution was analyzed for the following substances: Soluble nitrogen by a modification of Johnson's method (1941); glucose (residual reducing substance) by the method of Shaffer and Somogyi (1933); inorganic phosphorus by a modification of the procedure of Fiske and SubbaRow (1925); soluble carbon by a micro adaptation of the method of Friedemann and Kendall (1929).

Aliquots of the whole culture were analyzed for: ammonia nitrogen by the procedure of Gailey and others (1946); lactic acid by Barker and Summerson's method (1941); streptomycin by a modification of the method outlined by the Food and Drug Administration (1945) with pure streptomycin as a reference standard; oxygen demand ($Q_{O_2}$ per ml. of medium) was determined as described by Koffler and his co-workers (1945). The pH was measured with a glass electrode. A ten ml. aliquot was placed in a tared test tube and centrifuged. The collected mycelium was resuspended in distilled water to remove any remaining media and centrifuged a second time. The collected mycelium was then dried to constant weight at 100° C (12–16 hrs.); replicate determination agreed within 3 per cent. The dried mycelium was analyzed for nitrogen by Johnson's micro-Kjeldahl method (1941).

## EXPERIMENTS

The chemical changes occurring during the fermentation of the basal medium are presented in table 1. These analyses indicated that the medium

TABLE 1. *Changes Occurring During Fermentation of Glucose—Meat extract—Peptone Medium.*[a]

| | Duration of fermentation (days) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| pH | 7.35 | 7.30 | 7.55 | 7.50 | 7.75 | 8.25 | 8.55 | 8.65 | 8.90 |
| Mycelium mg./ml. | ..... | 0.4 | 5.1 | 5.8 | 5.7 | 4.8 | 4.6 | 4.2 | 3.8 |
| Streptomycin γ/ml. | ..... | 0 | 37 | 194 | 198 | 231 | 270 | 186 | 267 |
| Glucose mg./ml. | 9.0 | 8.8 | 8.0 | 2.4 | 1.2 | 0.6 | ..... | ..... | ..... |
| Soluble C mg./ml. | 10.2 | 8.6 | 7.0 | 5.1 | 5.0 | 4.4 | 4.6 | 4.5 | 4.6 |
| Lactic acid γ/ml. | 292 | 328 | 114 | 13 | 10 | 16 | 12 | 6 | 15 |
| Oxygen ($Q_{O_2}$/ml.) demand | ..... | 19 | 81 | 82 | 53 | 25 | −5 | ..... | ..... |
| Soluble N mg./ml. | 1.48 | 1.30 | 1.10 | 0.67 | 0.70 | 0.73 | 0.90 | 0.88 | 1.14 |
| Mycelial N % | ..... | 9.2 | 8.6 | 10.7 | 9.9 | 10.1 | 8.6 | 9.0 | 7.5 |
| Inorganic P γ/ml. | 118 | 108 | 34 | 1 | 5 | 2 | 19 | 24 | 34 |
| $NH_3$–N γ/ml. | 66 | 70 | 75 | 63 | 103 | 115 | 170 | 232 | 265 |

[a] Medium (per liter): 10 g. glucose; 5 g. meat extract; 5 g. peptone; 5 g. NaCl.

was very low in inorganic phosphate and that nearly all of the available phosphate was quickly assimilated by the organism. Accordingly a substantial quantity of inorganic phosphate was added to the medium to eliminate the possibility of this material acting as a limiting factor in the fermentation. Analyses of this supplemented medium are presented in table 2.

As the fermentable carbohydrate was very quickly utilized by the organism, it appeared possible that this might be a limiting factor in the fermentation. Various concentrations of glucose were added to the medium without marked effect on the production of streptomycin. It appeared that 1% glucose was nearly optimal under these fermentation conditions. The

TABLE 2. *Changes During Fermentation of High-Phosphate Medium.*[a]

| | Duration of fermentation (days) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| pH | 6.80 | 6.80 | 7.05 | 7.00 | 7.40 | 8.00 | 8.30 | 8.35 | 8.50 |
| Mycelium mg./ml. | ..... | < 0.1 | 3.1 | 5.2 | 2.2 | 1.7 | 1.4 | 1.5 | 0.8 |
| Streptomycin γ/ml. | ..... | 0 | 0 | 25 | 18 | 79 | 71 | 55 | 51 |
| Glucose mg./ml. | 10.0 | 9.0 | 7.8 | 2.0 | 0.5 | 0.2 | < 0.2 | ..... | ..... |
| Soluble C mg./ml. | 11.4 | 10.8 | 9.4 | 6.9 | 4.8 | 5.1 | 5.0 | 4.9 | 5.2 |
| Lactic acid γ/ml. | 212 | 185 | 48 | 45 | 45 | 32 | 23 | 13 | 7 |
| Oxygen ($Q_{O_2}$/ml.) demand | ..... | −10 | +117 | +104 | +54 | +30 | +22 | +20 | +8 |
| Soluble N mg./ml. | 1.04 | 0.95 | 0.85 | 0.59 | 0.90 | 0.93 | 0.85 | 0.91 | 0.88 |
| Mycelial N % | ..... | ..... | 6.0 | 8.3 | 7.7 | 8.5 | 9.5 | 13.0 | 12.5 |
| Inorganic P mg./ml. | 1.35 | 1.31 | 1.18 | 1.08 | 1.21 | 1.21 | 1.20 | 1.30 | 1.38 |
| $NH_3$-N γ/ml. | 78 | 44 | 33 | 18 | 95 | 226 | 306 | 290 | 305 |

[a] Medium: 10 g. glucose; 5 g. meat extract; 5 g. NaCl; 5 g. peptone; 5 g. $KH_2PO_4$.

changes occurring during the fermentation of a high carbohydrate (3 per cent glucose) medium are presented in table 3.

## DISCUSSION

The fermentation of this medium by *Streptomyces griseus* apparently falls into two phases as contrasted to the three phases during the *Penicillium chrysogenum* fermentation (Koffler *et al.* 1945). These general changes are summarized in table 4.

During the first or growth phase the mycelium is formed. This is accompanied by a marked reduction in the soluble nitrogen, the available inorganic phosphate, ammonia nitrogen, and fermentation of the available carbohydrate. During this phase the quantity of lactic acid present is first increased and then utilized to some extent. The oxygen demand is quite high during this first phase and $Q_{O_2}$ values of 150 have been observed. It is interesting to note that during the period of germination of the spores a gas is evolved, thus giving a negative oxygen demand. This has been repeatedly

TABLE 3. *Changes during Fermentation of High-Carbohydrate Medium.*[a]

| | Duration of fermentation (days) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 11 | 12 |
| pH | 6.55 | 6.55 | 7.40 | 7.10 | 6.65 | 6.90 | 6.90 | 6.75 | 7.00 | 6.70 | 7.65 | 7.85 |
| Mycelium mg./ml. | ..... | <0.2 | 3.3 | 7.1 | 5.5 | 4.9 | 4.5 | 4.8 | 3.8 | 3.6 | 3.6 | 3.1 |
| Streptomycin γ/ml. | ..... | 0 | 0 | 88 | 99 | 164 | 137 | 183 | 142 | 183 | 188 | 274 |
| Glucose mg./ml. | 30.0 | 29.0 | 27.0 | 23.0 | 19.0 | 16.0 | 10.6 | 8.5 | 4.5 | 2.5 | 2.0 | 1.5 |
| Soluble C mg./ml. | 19.8 | 19.6 | 17.0 | 14.3 | 14.0 | 12.2 | 7.5 | 6.7 | 6.3 | 6.5 | 5.7 | 5.9 |
| Lactic acid γ/ml. | 185 | 185 | 70 | 70 | 78 | 86 | 94 | 110 | 86 | 50 | 43 | 25 |
| Oxygen ($Q_{O_2}$/ml.) demand | ..... | −12 | +138 | +112 | +116 | +70 | +68 | +46 | +53 | +39 | +35 | +26 |
| Soluble N mg./ml. | 1.13 | 1.07 | 0.75 | 0.53 | 0.64 | 0.58 | 0.61 | 0.64 | 0.58 | 0.78 | 0.73 | 0.80 |
| Mycelial N % | ..... | ..... | 8.1 | 5.5 | 6.3 | 7.8 | 9.5 | 9.0 | 10.0 | 10.2 | 10.5 | 9.8 |
| Inorganic P γ/ml. | 153 | 122 | 60 | 27 | 18 | 28 | 30 | 28 | 27 | 4 | 6 | 3 |
| $NH_3$-N γ/ml. | 37 | 16 | 55 | 13 | 16 | <5 | 16 | <5 | 16 | 19 | 101 | 120 |

[a] Medium (per liter): 30 g. glucose; 5 g. meat extract; 5 g. NaCl; 5 g. peptone.

observed and the period extends until most of the spores have germinated. Although the gas has not been positively identified it is not presumed to be carbon dioxide as the Warburg flasks contained potassium hydroxide in the center well. The soluble carbon content of the medium during the growth phase rapidly falls as the glucose is utilized. Approximately 50 per cent of the soluble carbon of this basal medium was apparently not available to the organism during the fermentation. It appears that the nitrogen content of the mycelium varies with the age.

During the second or autolytic phase the bulk of the streptomycin is produced concurrent with a rise in the pH of the fermentation and a marked decrease in mycelium weight. The lactic acid content remains relatively constant during this phase as does the soluble carbon content of the

TABLE 4. *Changes Characterizing the Two Phases During the Fermentation.*

| | Phase I | Phase II |
|---|---|---|
| Streptomycin | Slight production | Maximum production |
| pH | Gradual rise | Reaches maximum |
| Mycelium | Rapid growth | Autolysis |
| Glucose | Rapid utilization | Small remaining amount exhausted |
| Soluble carbon | Gradual utilization | Reaches minimum and remains constant |
| Lactic acid | Slow production and utilization | Slow utilization |
| Oxygen demand | Maximum | Decreases to minimum |
| Soluble nitrogen | Used extensively | Concentration increases |
| Inorganic phosphorus | Used at maximum rate | Released into medium |
| Ammonia nitrogen | Rapid utilization | Concentration increases |

medium. The oxygen demand slowly decreases during this phase. In general the oxygen demand seems to parallel the utilization of the glucose. During the second phase the ammonia nitrogen, soluble nitrogen, and inorganic phosphate content of the fermentation rise rather markedly paralleling the autolysis of the cells.

Illustrative data of these changes are presented in table 1. When vegetative inoculum has been used for the fermentation the growth phase has been markedly shortened, but the general changes appear to be the same on a given medium.

As mentioned before, the basal medium appeared to be low in inorganic phosphate and various quantities of potassium phosphate were added to supplement that already present. The most marked effect of this supplementation was on the streptomycin production which was decreased as indicated in table 2. Perhaps the buffering action of the added phosphate accounted for the slower rise in pH during the fermentation.

When the carbohydrate content of the medium was increased to 3 per

cent the growth phase was apparently quite drawn out as indicated by the data of table 3. The pH rise was very much slower during the fermentation of this medium. The rate of glucose utilization apparently remained at a high value until most of the fermentable carbohydrate was utilized as indicated by the prolonged moderate oxygen demand. Whereas the mycelium did not autolyze markedly until most of the fermentable carbohydrate had been utilized, it appears that the quantity of available carbohydrate did not determine the quantity of mycelium formed. As might be expected the release of the inorganic phosphate, increase in ammonia nitrogen and increase of soluble nitrogen (usually found during the autolytic phase) did not occur until late in the fermentation. The rate of production of streptomycin during the fermentation was much lower on this high carbohydrate medium. Apparently some autolysis of the mycelium occurs as the nitrogen content of the mycelium reaches a rather high value.

The utilization of the soluble carbon of this medium is not well understood. The accumulation of volatile acids during the fermentation could not be detected. Woodruff and Foster (1943) noticed that a considerable quantity of lactic acid is formed by *Streptomyces lavendulae* during the fermentation of various media. The strain of *S. griseus* used in the experiments reported here also forms lactic acid, but to a much lesser degree. This lactic acid is apparently the *dl* mixture as identified by the hydration and rotation of the zinc salt, although on this meat extract medium no doubt most of it came from the meat extract. When certain synthetic media are fermented by this organism considerable quantities of lactic acid are formed and this is also the *dl* mixture. Apparently certain strains of *S. griseus* may utilize lactic acid (this will be elucidated in a later publication) and the strain used in these experiments belongs in this group. These experiments indicate that the glucose is utilized mainly as an energy source and not for mycelium formation. The character of the unavailable soluble carbon compounds present during the second phase of the fermentation has not been determined. These come from the peptone and meat extract and are not synthetic products, since in synthetic media all of the soluble carbon (supplied as glucose) disappears from the medium.

The production of streptomycin seems to be associated with the autolytic phase of the fermentation. Whether this indicates that streptomycin is synthesized during the latter phase of the fermentation or is a constituent of the mycelium has not been clearly shown. The experiments of Waksman and others (1946) may support the latter hypothesis. Determination of proteinase during the fermentation indicates that during the second phase the extracellular pH 7 proteinase increases.[1] Although the effect of high

[1] The proteinase determinations were made by following the hydrolysis of casein in a buffered medium by a method used by M. J. Johnson and associates.

phosphate in reducing streptomycin production might be attributed to toxicity, this concentration is no more than that present in cornsteep liquor media such as used by Schatz and his colleagues (1944).

SUMMARY

The chemical changes occurring during the fermentation of the Waksman-Schatz medium (1% glucose, 0.5% meat extract, 0.5% peptone, 0.5% sodium chloride) by a strain of *Streptomyces griseus* indicated that there are generally two phases of metabolic activity. During the growth phase the production of a mycelium is accompanied by a reduction in the soluble constituents of the medium (nitrogen, carbon, phosphorus), fermentation of the available carbohydrate, a high oxygen demand ($Q_{O_2}$ may reach 150), and little production of streptomycin. During the autolytic phase the mycelium weight decreases markedly, inorganic phosphorus and soluble nitrogen are released into the medium, the oxygen demand drops, and considerable quantities of streptomycin are produced. The pH rises gradually throughout the fermentation. Increasing the carbohydrate content of the medium has little effect on the general changes although it prolongs the growth phase. Increasing the phosphate content has little effect on the general changes but reduces the quantity of streptomycin formed. Carbon dioxide apparently is the main product of glucose utilization for detailed investigation has revealed no large accumulation of volatile or non-volatile acids. Some lactic acid is formed during the fermentation; this substance is also utilized by this organism.

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# SUPPLEMENTARY NOTES ON AMERICAN LABIATAE—IV

CARL EPLING

As material has accumulated through recent explorations, it has been my custom to supplement earlier revisions of American Labiatae by occasional notes describing new entities, giving noteworthy extensions of ranges, and modifying published keys to include new entities. These notes have previously appeared in this journal as follows: **67**: 509–536 (1940); **68**: 552–568 (1941); **71**: 484–497 (1944). The numbers preceding the specific names in the present paper are those used in the following appropriate revisions. **McClintock, E. & Epling, C.** A revision of *Teucrium* in the New World, with observations on its variation, geographical distribution and history. Brittonia **5**: 491–510. 1946. A review of the genus *Monarda* (Labiatae). Univ. Calif. Publ. Bot. **20**: 147–194. 1942. **Lewis, H.** A review of the genus *Trichostema*. Brittonia **5**: 276–303. 1945. **Epling, C.** Preliminary revision of American *Stachys*. Rep. Spec. Nov. Beih. **80**. 1934. The American species of *Scutellaria*. Univ. Calif. Publ. Bot. **20**: 1–146. 1942. Synopsis of the South American Labiatae. Rep. Spec. Nov. **85**: 1937. A revision of *Salvia*, subgenus *Calosphace*. Rep. Spec. Nov. **110**. 1939. A revision of *Hyptis* (ined.). **Epling, C. & Stewart, Wm. S.** A revision of *Hedeoma* with a review of allied genera. Rep. Spec. Nov. **115**. 1939.

## TEUCRIUM

3. T. CUBENSE Jacq. subsp. LAEVIGATUM McClintock and Epling. TEXAS: Hidalgo County, 5 mi. s.w. of Weslaco, *Tidwell 38*.

## MONARDA

8. M. LINDHEIMERI Engelm. and Gray. TEXAS: Gonzales County, Palmetto Park, *Albers 46099*.

11. M. PUNCTATA L. subsp. IMMACULATA Pennell. TEXAS: Bastrop County, Paige to McDade, *Tharp 32101*. Deep sand of the Carrizo in open woods, *Rodgers et al. 46529*.

12. M. FRUTICULOSA Epl. TEXAS: Kennedy County, Kennedy Ranch, *Tharp 42–40*.

14. M. CLINOPODIOIDES Gray. TEXAS: Travis County, Austin, *Warnock 245*. Hockley County, no locality, *Rachaner 132*.

17. M. CITRIODORA Cerv. TEXAS: Calhoun County, near Port Lavaca, *Gentry 45*. "Diminished form" referred to, p. 192.

## TRICHOSTEMA

7. T. BRACHIATUM L. TEXAS: Walker County, 4 mi. e. of Huntsville, *Albers and Warnock*.

STACHYS

3. S. ERIANTHA Benth. ECUADOR: Azuay: Paramos, vicinity of Toreador, 3810–3930 m., *Steyermark 53178.*

S. ? HAMATA Epl. VENEZUELA: Sucre: Cerro Turumquire, north-facing slopes between La Trinidad and zone of cloud forest, 1700–2000 m. *Steyermark 62510.* Very similar to this species, known only from the Paramo de Quindio, Colombia, but more lax and the trichomes elongate and scarcely pustulate at the base. In many respects resembles *S. nubilorum* Epl. of Guatemala.

SCUTELLARIA

6. S. AUSTRALIS (Fassett) Epl. TEXAS: Lavaca County, 3½ mi. n.e. of Hallettsville, *Webster 2013B.* Tyler County, 8 mi. w. of Woodville, *Rowell et al. 47213.*

26. S. WRIGHTII Gray. TEXAS: Williamson County, without locality, *Dyksterhuis et al. 16110.*

33. S. DRUMMONDII Benth. TEXAS: Kendall County, Edge Falls, *Tharp et al. 17T160.* Lee County, 1 mi. s. of Lincoln, *Rowell et al. 17M097.*

100. S. SPECIOSA Epl. What seems to be this species has been collected in PERU: Cuzco: Quispicanchi, Mandor; Marcapata, 1200 m., *Vargas 3811.*

102. S. ATRIPLICIFOLIA Benth. ECUADOR: Pichincha; wet luxuriant forested slopes or barrancos between kms. 37 and 50, along Rio Saloya, 1830–2430 m., *Steyermark 52534.*

106. S. VOLUBILIS Kunth. ECUADOR: Azuay: dry rocky slopes bordering Rio Leon towards Oña, 1970 m., *Steyermark 53701.*

109. S. BENTHAMIANA (Mansf.) Epl. PERU: Cuzco: Paruro, Huanoquite, *Vargas 2342.*

SATUREJA

S. LINEATA Epl. ECUADOR: Azuay: between kilometer 67 and south towards Oña, 3715 m., *Steyermark 53668.*

POLIOMINTHA

3. P. GLABRESCENS Gray. TEXAS: Presidio County, in creek bed in loose soil, Burnt Camp Canyon, the Solitario, 3800 ft., 30.XI.41, *Hinckley 2261.*

HEDEOMA

2a. **H. subaequale** Epl., sp. nov. Fruticulus altitudine ad 30 cm. ramulis gracilibus retrorse hirtellis; foliorum laminis ascendentibus anguste ellipticis etiam sublinearibus, 6–14 mm. longis, 1.5–3 mm. latis, in apice obtusiusculis sessilibus, integris, utrimque punctatis, glabris; cymulis plerumque 1-floribus; pedicellis gracillimis 6–9 mm. longis; calycum tubo 4.5–5 mm. longo, extus glabro, dentibus omnibus erectis anguste lanceolatis, acutissimis, 2.5–3 mm. longis, similibus, superioribus tamen quam inferiores paulo brevioribus, annulo sat denso; corollarum purpurearum tubo circiter 12 mm. longo intus pilis minutis crassiusculis sparse hirtello, vix tamen annulato.

MEXICO: San Luis Potosí. Rocky limestone, mountain above San Pedro, 2150–2200 m., 29.VII.1934, *Pennell 17731* (UCLA). Prope Morales in convalle San Luis Potosí, X.1876, *Schaffner.* San Luis Potosí, 1879, *Schaffner 412; 352.*

Allied to *H. apiculatum* Stewart, known only from the Guadalupe Moun-

tains of New Mexico and Texas (*Goodding 6023, Moore & Steyermark 3563*), from which it differs primarily in the conformation of the calyx and calyx teeth. This fruticulose section of *Hedeoma*, including also *H. hyssopifolium*, leans strongly toward the genus *Poliomintha*, from which it may be distinguished by the exannulate corolla tube.

7. H. MANDONIANUM Wedd. PERU: Cuzco: Paruro, Sullcan, Ayusbamba, 4020 m., *Vargas 887*.

16. H. REVERCHONI Gray. TEXAS: Llano County, near Enchanted Rock, *Barkley et al. 47307*.

17. H. ACINOIDES Scheele. TEXAS: Lee County, 1 mi. s. of Lincoln, *Bailey et al. 17M098*.

23. H. COSTATUM Gray. MEXICO: Hidalgo: 30 mi. n. of Zimapan in oak woodland, *Barkley 17M153*.

### SALVIA

10. S. BALLOTAEFLORA Benth. MEXICO: Tamaulipas: 20 mi. west of Reynosa, *Painter and Barkley 14392*.

21a. **S. Vargasii** Epl., sp. nov. (Tomentellarum) per specim. in Peruviae prov Cuzco prope Kairancka a Vargas (no. 5834) lectum constituta est; typus in herb. Univ. Calif. (Los Angeles).

Frutex sarmentosus altitudine ad 1 m. ramulis gracilibus minutissime hirtellis et glandulis sessilibus aureis conspersis internodiis quam folia plerumque brevioribus; foliorum laminis maximam partem 1–1.5 cm. longis, sat tenuibus, anguste ovatis, in apice obtusiusculis, in basi plus minusve truncatis, marginibus serratis, pagina superiore sparse hirtella, inferiore glandulis sessilibus aureis obtecta, petiolis gracilibus 3–4 mm. longis elatis; floribus paucis oppositis in racemis 2–3 cm. longis dispositis, bracteis caducis; calycibus florentibus 6 mm. longis, in maturitate paulo auctis, extus sparse hirtellis et glandulis sessilibus aureis conspersis, labia superiore curta mucronata; corollarum cyanearum tubo 14 mm. longo; staminibus subintegris, stylo minute hirtello fere glabro.

PERU: Cuzco: Grau: prope Kairancka, 2400 m., in declivibus aridis inter fructices, 9.III.1946, *Vargas 5834*.

Very similar indeed in aspect to *S. fruticulosa* of Oaxaca, but with notably larger flowers. At the same time its foliage recalls that of *S. Gilliesii* of the southern Andes, but much diminished. The stamens are almost entire, hence in the key to the sections it might be sought in *Flocculosae*.

57. S. CORRUGATA Vahl. ECUADOR: Azuay: between Milleturo and Toreador, 2590–3900 m., *Steyermark 53026*. Between km. 67 and south towards Oña, 3715 m., *Steyermark 53673*.

145. S. HIRTA Kunth. ECUADOR: Azuay: dry rocky slopes bordering Rio Leon towards Oña, 1970 m., *Steyermark 53708*.

160. S. UNCINATA Urb. SANTO DOMINGO: San Juan: Sabana Nueva, 6200 ft., cordillera central, n. of Rio Arriba del Norte, *R. A. and E. S. Howard 9155*. Apparently this, but leaves lanceolate and elongate; no mature flowers.

164. S. THORMANNI Urb. SANTO DOMINGO: San Juan: pine woodlands, Piedra del Aguacote to Rio del Oro, 5200 ft., *R. A. and E. S. Howard 9382*. Apparently this, but no flowers. Said to be vine-like.

167. S. COCCINEA Juss. TEXAS: Brownsville, *Shiller 864; 637*. A color variant in which the corollas appear to be of a more bluish red. *Fisher 41034*.

201. S. (?) SARMENTOSA Epl. Peru. Apurimac. Grau: Cuesta de Seccsecka in declivibus inter frutices, 3800 m., 4.III.1946, *Vargas 5763*. Vargas' specimen is very similar to one gathered by Stafford in Urcos valley, Cuzco, save only in the notably larger flowers (calyces 11 mm. long, larger at maturity; corolla tube 12 mm. long). I previously referred the latter to *S. sarmentosa*, described from Ollantaytambo, despite differences in pubescence and habit. It is difficult to say whether two species are actually represented. In any case, both forms find their closest alliance with *S. lycioides* and *S. Greggii* of northeastern Mexico.

204. S. LYCIOIDES Gray. TEXAS: Presidio County, Chinati Mts., about 10 mi. w. of Shafter, *Hinckley 3538*.

226. S. SPHACELIFOLIA Epl. MEXICO: Sinaloa: Sierra Surotato, Canyon de Tarahumare, 3000–4000 ft., mixed subtropical vegetation with lower oaks. *Gentry 7295*. Sierra Surotato, Los Pucheros, 5500–6500 ft., pine-oak-madrone, open forested slope. *Gentry 7209*.

239. S. AMISSA Epl. ARIZONA: Graham County, on rocky shaded canyon slope; 3200 ft. elev.; 10 mi. n.w. of Klondyke in Aravaipa Canyon. *Darrow*.

242. S. FARINACEA Benth. TEXAS: Shelby County, 12 mi. n.w. of Center, *Lee 104*. McLennan County, near Waco, *York 46143*.

249. S. MALACOPHYLLA Benth. ECUADOR: Loja: between S. Pedro and Chinchas, 1600 m., *Espinosa 1297*. Originally described from Mathew's collection near Sesuya, Peru and not since collected. *S. loxensis* known only from Hartweg's collection near Loja is similar but glabrate. It may be only a variant. If so the latter name has priority, but both may be conspecific with *S. mitis* R. and P.

305. S. TILIAEFOLIA Vahl. TEXAS: Presidio County, street in Marfa, 17.X.1942. *Hinckley 2636*. First record for the United States. Arizona. Cochise County, deep shade of limestone cliff, 5500 ft., Mustang Mts., near Harrison Ranch, *Darrow 3628;* apparently indigenous, according to the collector.

306. S. OBVALLATA Epl. COLOMBIA: Cauca: Cord. Central vertiente occ. Hoya del rio Palo, matorrales y bosques subseriales en La Tolda, arrica de Tacueyo, 2000–2030 m. 18.XII.1944. *Cuatrecasas 19409*.

344. S. (?) AMPELOPHYLLA Epl. VENEZUELA: Merida: Paramo de los Colorados, between El Molino and San Isidro Alto, 2745–2955 m., *Steyermark 56540*. Moist rich woods, Los Quebraditos, above Juji, 2509 m., *Steyermark 55997*.

This species is known from several collections in El Cauca. Whether the present collections are referrable to it is uncertain. The flowers are smaller (corolla tube 12 m. long), pale blue or white, and the corolla tube is not invaginate. Yet the habit and aspect of the rather poor material is remarkably similar. In the key to the sections, these specimens would be sought under section *Angulatae*, because of the short tube.

365a (177). S. ORBIGNAEI Benth. Recent collections by Cardenas permit an emendation of the characters of this species. The stamens are included within the upper lip, and not exserted, as indicated in the sectional description. In pressing, they frequently become extruded and give that appearance. The lower lip is shorter than the upper, or at most subequal. Although the base of the corolla tube constantly bears two well defined papillae within, the degree of invagination is variable. White-flowered forms may occur accord-

ing to Cardenas. For these reasons, it seems desirable to assign *S. Orbignaei* to sect. *Pavonia* as no. 365a, amending that section to include simple as well as branched hairs, since both may occur in *S. Orbignaei*, and to assign *S. perlonga* to a new section, *Nelsonia* (see below). The amended key to the section *Pavonia* would read as follows:

| | |
|---|---|
| Corollarum tubi 13–14 mm. longi. | 364. *S. revoluta.* |
| Corollarum tube 19–24 mm. long. | |
| Folia in basi truncato-subcordata; plantae peruvianae. | 365. *S. Macbridii.* |
| Folia in basi angustata; plantae bolivianae. | 365a. *S. Orbignaei.* |

Following are Cardenas' collections: BOLIVIA: Cochabamba: Morochata, 2900 m., *Cardenas 2497;* Angostura, 2560 m., *Cardenas 2396;* Orami, 2700 m., *Cardenas 2395;* Ansaldo, 3000 m., *Cardenas 2447.*

39. **Nelsonia** Epl., sect. nov.

Suffrutices foliis oblongis rugosis; calycum labia superiore 5-venis paulo longiore; corollarum coccinearum vel alarum sat crasso ventricoso ad basim constricto-invaginato et intus rugis binis obliquis ornato, labia superiore quam inferior longiore; staminibus e galea 3–4 mm. exsertis, ad fauces positis; jugo ad connexum in dentem parvum producto; styli villosi ramo postico longiore. Plantae Mexicanae; species typica est *S. perlonga.* The key to the sections should be amended to read as follows:

p. 6 line 7: "EE. Corollarum tubi fere recti ad basim sub papillas invaginati; stamina ad fauces posita. 39. *Nelsonia.*"

p. 11 line 31: "I. Pili ramosi. J. Plantae peruvianae vel bolivianae; corollarum tubi 13–24 mm. longi. 64. *Pavonia* (vide etiam *Cardinales*)."

p. 12 line 6: "L. Corollae ad basim valde invaginatae nullomodo lateraliter saccatae. M. Folia ampla in basi rotundata vel cordata; plantae brasilianae. 76. *Hoehneana.* MM. Folia oblonga in basi angustata. 64. *Pavonia.* LL. Corollae ad basim ——."

368a. **S. iuliana** Epl., sp. nov. (65. *Nobiles*)

Herba perennis ramosa altitudine ad 2 m., ramis hirtellis, foliorum laminis membranaceis 5–6 cm. longis, 2–2.5 cm. latis, ellipticis utrimque acuminatis, marginibus serratis, paginis ambabus glabris nisi sparsissime hirtellis, petiolis ad 1 cm. longis elatis; floribus (in specimini suppetenti) in foliorum axillis solitariis, pedicellis gracilibus ad 15 mm. longis elatis; calycibus florentibus circiter 22 mm. longis, in maturitate paulo auctis, labiis acuminatis subaequilongis 8–9 mm. longis superiore sub-5-venis, inferiorum dentibus omnino connatis; corollarum coccinearum tubo circiter 30 mm. longo, ad basim paulo constricto, labia superiore circiter 18 mm. alto, inferiore breviore. VENEZUELA: Merida: Cuesta de Rincon and Cuesta del Barro, between Canagua and La Quebrada, on road to El Molino, 1925–2175 m., May 10, 1944, *Steyermark 56450* (type, U.C.L.A.).

The large-flowered sections *Nobiles, Longiflorae,* and *Tubiflorae* are closely allied. The two former are separable chiefly by the habit of the foliage, the latter is distinguished by the usually 3-veined upper calyx lip. Steyermark's accession appears to be more nearly allied to *Nobiles* and is similar in many ways to *S. balaustina* of east central Brazil.

420. S. LATENS Benth. VENEZUELA: Merida: dry rocky gneissic-granitic slopes between Timotes and Paramite, 2285–3500 m., *Steyermark 55700.* Apparently this, known previously only from Colombia.

443d. **S. anguicoma** Epl., sp. nov. (87. *Purpureae*). Herba suffruticosa altitudine ad 2 m., ubique pilis crassioribus anguicoma; foliorum laminis ovato-ellipticis 5–7 cm. longis, 2.5–3.5 cm. latis, in apice breviter acuminatis, in basi ad petiolos saepius 2–4 cm. longos cuneato-angustatis, marginibus serratis, venis subtus prominulis; floribus plerumque tribus in verticillastris; calycum florentium tubo 5–6 mm. longo, labiis subaequilongis, dentibus acuminatis; corollarum purpurearum tubo circiter 12 mm. longo. VENEZUELA: Merida: Woods above Las Cuadras, 1520–2285 m., March 30, 1944, *Steyermark 55830*.

Following is a revised key to *Purpureae* (p. 329):

Plantae colombiano-venezuelanae.
- Folia utrimque pilis crassioribus villoso-anguicoma. 443d. *S. anguicoma.*
- Folia subtus molliter vel tomentosa vel pubescentia, interdum glabrata.
  - Corollarum tubi plerumque 10–12 mm. longi.
    - Ramuli molliter tomentosi; calyces lanata. 443. *S. sordida.*
    - Ramuli plus minusve villosi; calyces glabrata. 443c. *S. Cuatrecasana.*
  - Corollarum tubi 15–16 mm. longi.
    - Calyces florentes 7.5–8 mm. longi; folia subtus molliter pubescentia. 444. *S. rufula.*
    - Calyces florentes 9–10 mm. longi; folia subtus dense molliterque tomentosa. 445. *S. tolimensis.*

Plantae boreali-americanae.
- Folia subtus solum ad venas pilis crassioribus villosa; calyces 11 mm. longi. 441a. *S. Matudae.*
- Folia subtus pilis gracilibus modo pubescentia vel tomentosa modo glabrata; calyces 4–8 mm. longi.
  - Petioli plerumque 2–4 cm. longi et ultra, folia in basi plerumque rotundata.
    - Corollae extus pilis crassis dense villosae; tubi fauces 3–6 mm. diametro. 441. *S. Littae.*
    - Corollae extus pilis tenuibus pubescentes; tubi fauces 1.5–3 mm. rarius 4 mm. diametro. 442. *S. purpurea.*
  - Petioli plerumque 1–1.5 cm. longi.
    - Corollarum tubi 18–22 mm. longi. 440. *S. curviflora.*
    - Corollarum tubi 10–12 mm. longi.
      - Folia subtus tomentosa, superne areolato-hirsuta. 443a. *S. areolata.*
      - Folia utrimque praesertim ad venas hirtella. 443b. *S. sparsiflora.*

## HYPTIS

1a. **H. cuniloides** Epl., sp. nov. (1. *Umbellaria*) Fruticulus altitudine circiter 20 m., ramulis incano-puberulis; foliorum laminis fasciculatis 2–3 mm. longis, plerumque obovatis, integris sessilibus, pilis minutissimis argenteis; cymulis 3-floribus in foliorum supremorum pedunculis 5–6 mm. longis elatis; calycibus florentibus glanduloso-villosulis, in maturitate 4 mm. longis, dentibus deltoideo-subulatis vix 1 mm. longis; corollarum tubo circiter 6 mm. longo, superne sat ampliato; gynobasi non viso; nuculis ut videtur laevibus. BRAZIL: Bahia: Mun. Morro do Chapeu, sandy soil, treeless "subalpine" zone, 1000 m., with *Velloziaceae* etc., April 1944, *Schery 587* (type, Mo. Bot. Gard.).

Resembles *Cunila incana*. Apparently referable to section *Umbellaria*, but differs markedly in aspect, perhaps because of the minute leaves. The character of the nutlets and gynobase could not be clearly discerned.

59. H. FLORIBUNDA Briq. ARGENTINA: Santa Fe: Lauteri, depto. Gral, Obligado, *Maldonado 1716.*

142. H. LACINIATA Benth. COLOMBIA: Meta: edge of thicket, 200 m., sandy flood plain of Rio Muco, 70 km. s.e. of San Pedro de Arimena, *Hermann 10932.*

143. H. ULIGINOSA St.-Hil. BRAZIL: Rio Grande do Sul: San Salvador, *Eugenio Leite 751.*

147. H. MICROPHYLLA Pohl. VENEZUELA: Bolivar: Tumeremo, 305 m., *Steyermark 60923.*

269. H. OBTUSIFLORA Presl. ECUADOR: Loja: Torata, *Espinosa 1190.*

DEPARTMENT OF BOTANY, UNIVERSITY OF CALIFORNIA
LOS ANGELES, CALIFORNIA

BULLETIN OF THE TORREY BOTANICAL CLUB

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# TORREYA

## FIELD TRIP REPORTS

MAY 11. PERKIOMEN VALLEY, MONTGOMERY COUNTY, PA. This was a joint outing through the courtesy of the Academy of Natural Sciences of Philadelphia. Torrey Botanical Club members were a distinct minority but none the less interested and appreciative. The outing included a stop at Kibblehouse Quarry, Perkiomenville, where the geology was explained and identification of various rocks was made by Dr. E. T. Wherry. Nice stands of *Viola striata* and *Mertensia virginica* were discovered along Perkiomen Creek adjacent to the quarry. A further fine display of spring flowers was found along Unami Creek near Sumneytown, most noteworthy being *Cercis canadensis, Viola triloba, V. conspersa, Castilleja coccinea, Aquilegia canadensis,* and *Caltha palustris.* Total attendance 78. Leader for Torrey Botanical Club, Louis E. Hand.

MAY 30–JUNE 1. TANNERSVILLE, PA., in the Poconos. Participation in this weekend was also through courtesy of the Academy of Natural Sciences of Philadelphia. The program opened with a trip to Big Pocono Mountain. Enroute, our guide from the Pennsylvania Department of Forestry showed the group what is considered the largest *Tilia americana* in the United States. It measures 20 ft. 7 in. in circumference at the ground, 16 ft. 3 in. at breast height, is 104 ft. 9 in. tall, and has a spread of 75 ft. Its age is estimated at 350 years. The summit of Big Pocono (2,131 ft.) afforded excellent views but little difference in vegetation from the Tannersville region. *Rhododendron roseum* Rehder, the northern, mountain relative of our coastal plain *Rhododendron nudiflorum,* and called *R. canescens* in Gray's Manual, was the outstanding floral attraction. It was found in abundance and at the height of bloom. Other trips included a fossil coral reef at Analomink, the vicinity of Buck Hill Falls Inn, Tannersville bog, and Swiftwater Falls. Plants of northern affinity encountered included *Kalmia polifolia, Andromeda glaucophylla, Picea mariana, P. rubra, Larix laricina, Eriophorum callitrix, Vaccinium Oxycoccus, Carex limosa, Calla palustris,* and *Fraxinus nigra.* Attendance over 100 (13 Torrey Botanical Club members). Leader for the Club, Louis E. Hand.

JUNE 14–15. DELAWARE WATER GAP, PENNA.–N. J. Saturday was devoted to a hike along Kittatinny Mtn. and over Mt. Tammany from where panoramic views from the rocky outlook were enjoyed by all. A botanical survey list was compiled for this newly opened section of the Appalachian Trail. The Sunday hike was also along the A. T., ascending Mt. Minsi on the Penna. side of the gap. General opinion was that views from Tammany excel. Attendance 9. Leader, Louis E. Hand.

JUNE 15. MILLBURN, N. J. "This was a pleasant little trip through the various paths and bridle paths of Sounth Mountain Reservation where many of our common flowers were noted, particularly *Krigia amplexicaulis* and *K. virginica,* the former abundant throughout and the latter in a fair stand on rocks. The highlight of the trip, botanically, was the 'Blazing Star,' *Chamaelirium luteum* (L.) Gray which is not recorded in Essex County, N. J. by Norman Taylor's Flora of the Vicinity of New York." Attendance 10. Leader, William Rissanen.

JUNE 21–22. FORKED RIVER, N. J. Many new species were added to our list from this locality. The most spectacular find was *Listera australis,* of which a stand of eight plants was found. One specimen was taken for deposit in the herbarium of the Academy of Natural Sciences at Philadelphia where the other known specimens from N. J. are deposited. Something of a record may also have been set by David Fables who drove from the Gaspe directly to Forked River for this trip. Attendance 9. Leaders, Louis Hand and Hollis Koster.

JUNE 22. ARDEN CIRCULAR IN PALISADES INTERSTATE PARK. A small group with much energy lengthened the walk somewhat. Plants reported to be of interest at the time were *Rhododeneron maximum* (apparently nearly a month from flowering), *Calla palustris*, and *Sarracenia purpurea*. Attendance 3. Leader, Dr. H. B. Gordon.

JULY 12. WEST ORANGE, N. J. Approximately 100 species and varieties of ferns and fern allies were growing in the garden of our leader at this time. They attracted much attention and favorable comment. Attendance 18. Leader, W. H. Dole.

JULY 13. HAMDEN, CONNECTITUT. Torrey Botanical Club people were again in the minority, this being a joint outing with the Connecticut Botanical Society. Those present expressed much interest in the chestnut plantations. Attendance 40. Leader, Dr. A. H. Graves.

JULY 19. WARD'S POINT, STATEN ISLAND, N. Y. "We were glad to note that the two local celebrities in the botanical line were still there. *Allionia nyctaginea* and *Polanisia trachysperma* seem to hold their own. In addition we found a third rather rare adventive: *Lythrum alatum*. I found a single plant of this a few years ago but now there was a colony of several dozen. Among the more usual plants worth mention were *Asclepias tuberosa*, fairly abundant here though it is said to be becoming rare on the Island. Several Euphorbias grow here including *E. Cyparissias* and *E. polygonifolia* as well as the ubiquitous *E. maculata*. *Convolvulus sepium* and *C. arvensis* were in evidence also. Among the trees of interest were *Morus alba* with fruit in just the right condition for sampling, *Populus alba*, and *Salix alba*, *Broussonetia papyrifera*, and *Celtis occidentalis*." Attendance 12. Leader Mr. Charles Ericson.

AUGUST 2–3. MONTAUK POINT, LONG ISLAND, N. Y. "We were especially honored and happy to have with us Dr. John H. Barnhart, in point of years of membership, the oldest living member of the Torrey Botanical Club. His genial presence added much to the enjoyment of the trip. We were also fortunate in having Mr. E. J. Alexander with us, who showed us many extremely interesting and rare plants which we would otherwise have missed, and who checked our identifications." Attendance 23. Leader Dr. H. N. Moldenke.

AUGUST 10. QUARRY LAKE, VALHALLA, N. Y. This trip, through the courtesy of Mr. Nathan Straus, included a tour of the estate and study of the many interesting plantings. Attendance 15. Leaders Mr. Ernest Hoelle and Dr. Harold N. Moldenke.

AUGUST 24. SEELEY'S NOTCH, SCOTCH PLAINS, N. J. This walk through the Watchung Mountains provided a great number of the plants to be expected in such terrain, and lists of these have been published in seasons past. Newark Museum Nature Club members and friends were our guests. Attendance 23. Leader, Dr. Harold N. Moldenke.

AUGUST 20–29. MONTREAL AND THE GASPÉ REGION, QUEBEC. Joint affair with the Botanical Society of America and the American Society of Plant Taxonomists in connection with the establishment of a Northeastern Section of the Botanical Society of America. The first two days were given over to lectures, symposia, and miscellaneous papers together with exploration of the remarkable Jardin Botanique. On Friday a trip to Morgan's Woods gave an intimate view of the deciduous forest as represented north of Montreal. A carefully prepared and fully documented tour guide for the Quebec-Gaspé excursion arranged by Dr. Pierre Dansereau and Mr. Marcel Raymond, was fully appreciated as the days ensued. Since this is to be revised and made available generally, the present report will note only the places visited. A surprisingly large percentage of the characteristic species listed in the tour guide were seen at each stop by those who looked carefully. Many additional species were turned up in the process of combing the various localities and referred to the leaders for identification or confirmation. The presence of Mr. Ernest Rouleau, Curator of the Marie Victorin Herbarium of the Institute Botanique of the University of Montreal was an added reassurance. Places visited and their objectives follow: Cap-de-la-Madeleine, pine barrens (substitute *P. rigida* for *P. banksiana* and one would think himself back in Jersey, for the most part). Sainte-Anne-de-la-Perade, Typical St. Lawrence lowland forest vegetation. Neuville, fresh-water intertidal zone (shingle beach and ledges).

Duchesnay, deciduous forest and coniferous forest more or less intermixed (overnight at Forest Rangers School). Saint-Vallier, deciduous forest, intertidal zone, tidal pools and other estuarian elements but not fully salt water. Sainte-Anne-de-la-Pocatiere, grounds and adjacent forest of a school of agriculture. Kamouraska, sufficient stops in the vicinity to impress the geological and botanical formations of this name upon everybody, particular attention given to the marshes and their zonation. Saint-Fabien, raised bog (with an extra one thrown in en route). Le Bic, salt marsh, silty intertidal area, and rocks. Baie-des-Sables, shore zonation (*Mertensia maritima* and *Senecio Pseudo-Arnica* got most of the attention). Sainte-Anne-des-Monts, the wonderful sequence of forest, scrub, alpine meadow, ravines, and stony summit of Mt. Albert is beyond description. Mont-Saint-Pierre, plants of the shifting schists. Perce, endemic and other plants of exposed cliffs and crevices together with the more extensive communities of grass and woodland. Paspebiac, sand bar vegetation. Bonaventure River flats, just that but with some nice endemics and more widely spread species. Salt marshes were also studied here. Attendance, over 100 with 80 odd making the tour in 23 cars. Leader, Dr. Pierre Dansereau.

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COMPILED BY

LAZELLA SCHWARTEN

WITH THE COLLABORATION OF THE EDITOR OF THE TAXONOMIC INDEX

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**Smith, Gilbert M.** The historical significance of names applied to reproductive structures of algae. Farlowia **3**: 217–223. Jl [Au] 1947.

**Taft, Clarence E.** *Spirogyra Hollandia*, a new species from New Guinea. Ohio Jour. Sci. **47**: 173. *f. 1.* Jl [Au] 1947.

### FUNGI AND LICHENS

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**Long, W. H. & Ahmad, Sultan.** The genus *Tylostoma* in India. Farlowia **3**: 225–267. *f. 1–25.* Jl [Au] 1947.

**Shear, C. L.** Studies of types and authentic specimens of *Hypoxylon*—II. Lloydia **10**: 60–63. *f. 1, 2.* Mr [Je] 1947.

**Singer, Rolf.** Coscinoids and coscinocystidia in *Linderomyces lateritius.* Farlowia **3**: 155–157. *f. 1.* Jl [Au] 1947.

**Vanterpool, T. C.** *Selenophora linicola* sp. nov. on flax in Saskatchewan. Mycologia **39**: 341–348. *f. 1–10.* My 1947.

**Wilson, Charles Maye.** Coprophilous Ascomycetes in Virginia. Mycologia **39**: 374–377. My 1947.

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**HERE** Dual-Cone Nosepiece. Twice the usual bearing surface. Always correctly aligned.

**HERE** Mechanical Stage. Chrome against brass bearing action. Binding is eliminated.

**HERE** Fork-Type Substage. Condensers interchanged quickly and easily. Positive alignment.

## Distinguishes Spencer Medical Microscopes

Precision design of a microscope stand is as important as the quality of optics. Spencer No. 33 Microscope (above) and Binocular Microscope No. 13 (right) incorporate many mechanical features for durability and convenient examination of specimens.

*For further information write Dept. Y74.*

American Optical Company
Scientific Instrument Division
Buffalo 15, New York

*Manufacturers of the* SPENCER *line of fine instruments*